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Dissertation

THE DEVELOPMENTAL ANATOMY OF THE HEAD OF THE BLOWFLY LARVA, CALLIPHORA ERYTHROCEPHALA (MEIGEN 1826)

by

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INTRODUCTION

The morphology of the cyclorrhaphous fly larvae, more commonly referred to as the "acephalous" fly larvae, has been the subject of research for over a century; and yet there exists today not one standard reference which gives a detailed account of the anatomy of the head region. Not even the fruit fly, the "guinea pig" of the geneticist, can claim such an honor. It would seem logical to suppose that before any experiments were to be performed on an organism, as for instance grafting, transplantation, or extirpation, the exact morphological nature of the organism would be investigated and made known. Yet this is hardly the case, as will be pointed out in this investigation.

It is undoubtedly a fact that very few biologists today, especially morphologists, would consider themselves as Lamarckians or even leaning toward Lamarckism in their explanations of morphological principles. Yet even at the present time in the field of insect morphology there appear explanations which are entirely out of step with the concept of evolution as revealed by the study of genetics for the past fifty years.

INTRODUCTION

One such explanation can be discerned in the status of the cyclorrhaphous larval head as interpreted by Snodgrass, one of the eminent insect morphologists of the present day. His conclusions are drawn from studies of the heads of the muscoid larvae, which belong to the larger group, the Cyclorrhapha. According to him, the head of the muscoid larva is invaginated and the sclerotized apparatus which develops on the walls of this invagination bears no relationship to the head capsule of other insects. Furthermore, the mouth hooks possessed by this larva are claimed by him to be entirely new structures developed from a part of the body wall other than the head. In other words, the muscoid larval head is an entirely new development, derived for the benefit of the larva and bearing no relationship to the head of any other insect, not even the other dipterous larvae ! Here is an example of disorder in evolution, the type of evolution as exemplified by Lamarckism. Here we have new structures arising as needed by the animal without regard for any pre-existing structures. This denies the theory that evolution is an orderly process initiated by the internal environment of living material.

One very obvious fact which has evolved from the study as presented in this paper is the "isolation policy" held by many entomologists concerning the fields of

embryology and morphology, in that these two related studies are so often considered to be apart from one another. It is a rare case indeed where an embryological study is taken through the hatching stage and into the next stage of development, or where a morphological study is started from its real beginning in the embryo. Certainly the morphology of an organism has its beginning in the embryology of that organism. There exists, at least in entomology, a gap between these two branches of study that needs to be bridged. This gap exists in the literature, but most certainly not in the organism. It is a falacy to believe that the embryology of a blowfly, for instance, ceases with the hatching, since metamorphosis is but delayed or suppressed embryology.

As will be readily noted in the presentation of the literature, insect embryology, especially of the Diptera, has received but little attention. Most of our present knowledge concerning the embryology of insects comes from work accomplished during the latter part of the last century. Moreover, when examined more closely, it becomes obvious that the embryology presented is mainly early embryology, that is, up to but not including morphogenesis. Those few papers that do give some late development, especially on the head region, are quite sketchy and inadequate. It has also been noted that many of the

embryological studies which have been made since the beginning of the twentieth century have taken, a priori, the work of the earlier embryologists. Certainly our improved equipment and technique warrants a reinvestigation of the work done earlier when methods, and particularly equipment, were inadequate for embryological studies on eggs less than one millimeter long.

The morphological studies of the insect have been exceedingly numerous and text books have been published accumulating the data. However, much is yet to be desired, as evidenced by the conclusions noted above concerning the nature of the head of the cyclorrhaphous larva. The principle that evolution is an orderly process was hardly followed in that case. It is also strongly felt that some workers in insect morphology, as evidenced by descriptions of observations made, do not have a firm grasp of the basic fundamentals of insect anatomy. A few examples of this will be noted in the discussion.

Unfortunately, most of the embryological and morphological studies of insects have not been made with definite principles in mind, as has been done with the vertebrates, for example. As a consequence, the entomological literature is cluttered with individual pieces of research,

few of which contribute anything toward a correlation of the evolutionary processes involved among the Insecta.

About ten years ago, Ferris of Stanford University realized the need of conscientiously applying various principles and hypotheses in studying the comparative anatomy of the insects and of the larger group known as the Annulata. In the section concerning the literature, Ferris' work and that of his students will be mentioned and quoted. The present study was carried out with the same principles in mind.

Before stating the problem and scope of this paper, a few statements will be made concerning its development. Approximately ten years ago, when the topic of the day among entomologists was the hormonal control of growth and development in insects, it was decided by the writer to make a study of the embryology of the ring gland in the blowfly, Calliphora erythrocephala (Meigen). This ring gland was suspected of playing a role in the process of hormonal control, and its exact makeup or morphology was not known. As the study progressed and development of the head region was followed in general, it became apparent that few landmarks existed for orientation. A review of the literature failed to shed any light on the subject; instead, it brought to light the confusion that existed

concerning the embryology of the cyclorrhaphous larval head. Further study of the embryological material at hand revealed facts about the development of the head which were not in agreement with the regularly accepted viewpoint. Going further, the accepted viewpoint on the development of the head naturally had its influence on the morphological studies, and a review of the morphological literature on the nature of the cyclorrhaphous larval head revealed just this fact. The review revealed that, because of the way in which the head developed, it could not possibly bear any relationship to the usual conception of the insect head. Hence here was something new, developed especially for the maggot, with no regard for pre-existing structures--a truly Lamarckian attitude ! It became obvious that a plausible explanation of the ring gland could not be attained. The problem of the embryology of this gland now became completely over-shadowed by the much more fundamental and basic problem of the development of the head and its morphological structure in correlation with our present knowledge of the insect head.

The present investigation was undertaken to establish the morphology of the head of the blowfly larva, Calliphora erythrocephala, and to substantiate the morphological findings with embryological evidence. Gross dissections

were made in working out the musculature, innervation, and the sclerotized parts. Histological sections were made to verify the nature of tissues involved and for purposes of orientation. They were also used, on occasion, to verify muscle origins and insertions. However, histological sections were found unreliable in reconstructing the nervous system. In all cases, gross dissection proved much more accurate than histological sections. Although not illustrated or discussed in the main body of this paper, dissections were made of larvae of Musca domestica L., Lucilia sp., and Drosophila melanogaster Meigen for comparison. No major differences occurred.

This study was made with the idea that the course of evolution is epigenetic and that every structure developed has its precursor. Existing structures were homologized as much as possible, using the trends as observed by Cook (1949) in his study of the evolution of the head in dipterous larvae. Names were not given to structures just for the sake of naming them. Where reasonable explanations could not be given for particular structures involved, those structures were left unnamed. A complete picture of the embryology of the head was not intended and is not presented.

TECHNIQUE

A culture of blowflies was started by placing some beef outside and waiting for the deposition of eggs by the proper species of fly. Once obtained, the eggs from a single female were brought into the laboratory and placed in a prepared fly cage. The cage was approximately 2' x 1½' x 1', with a wooden base and a glass front for observation. The remaining sides were covered with muslin. One end had a large, funnel-shaped piece of muslin for the feeding and handling of the flies. The cage was placed in a hood with a suction fan, which could be turned on to get rid of any offensive odors.

The life cycle at room temperature was completed in about 14 days; the eggs hatched in 21 hours, the larval stage lasted about six days, and the pupal stage about seven days. Moist sand in a battery jar was provided the larvae for pupation. Once the culture was underway, water and sugar were kept in the cage at all times. To stimulate the production of eggs, meat was added a few days in advance. About 30 to 50 flies were kept in the cage. Whenever the number got down to about 12 (due to mortality), a new culture was started.

The eggs were fixed in alcoholic Bouin's (Galigher,1934)

or in Dietrich's fluid (Kingsbury and Johannsen, 1935), both of which gave good results. Dietrich's fluid, when used warm, gave faster penetration and better fixation. For proper fixation the chorion was removed and the vitelline membrane pierced at the posterior end while the egg was in the fixing fluid. Dechorionation was easily accomplished by rolling an ordinary dissecting needle over the surface of the egg until the chorion split. Then, by proper manipulation of the needle, the egg popped out of the shell. The eggs were kept in the fixing fluid for 24 hours and then dehydrated to 85 per cent alcohol, in which they were kept until needed.

For purposes of orientation in the embedding medium for sectioning, it was found helpful to place the eggs in a 95 per cent alcololic eosin solution before clearing. This gave them a slight tinge of red which greatly facilitated the whole embedding procedure. The eggs were sectioned at seven micra. Longitudinal, both front and sagittal, as well as cross sections were made. The staining procedure employed was that found in Galigher (1934), using Delafield's hematoxylin and counter-staining with eosin. Another staining procedure used to great advantage, especially for the nervous system, was the protargol method (Bodian, 1936).

First, second, and third instar larvae were fixed in warm Dietrich's fluid and left in the fixative for 24 hours, during which time the posterior end was cut to insure fixation. They were then dehydrated to 85 per cent alcohol, and left in the fluid until needed. The larvae were imbedded in rubber paraffin and sectioned at 8 micra. They were stained with Delafield's hematoxylin and counter-stained with eosin.

Gross dissections of all larval instars were made, both on preserved and fresh material. Dietrich's fluid and 95 per cent alcohol were both employed as fixatives, the former being far superior in all respects. Larvae killed in Dietrich's fluid and preserved in 85 per cent alcohol have been opened and dissected after five years and found to be perfectly preserved.

Syracuse watch glasses, partly filled with waste paraffin, were used as dissecting trays. The larva was pinned in place, cut open with iridectomy scissors, pinned out, and covered with water. In dissecting it was found helpful to employ a stain to bring out the muscles and nerves. Standard alum hematoxylin (Galigher, 1934) proved best. About four or five drops of the stain were mixed with the water covering the larva, allowed to remain

for about five minutes, and then replaced with clean water. With this technique, the muscles and nerves were easily recognized and followed.

The dissection of fresh material was far superior to the preserved, especially in respect to the nervous system. The living larva was pinned to the paraffin in a somewhat stretched state, and an insect physiological saline solution was poured over it. The larva was then slit open and the hypodermis was pinned out. The saline was poured off and a 1:1,000 solution of methylene blue in physiological saline was added, covering the larva. The dish was gently agitated from time to time to mix air into the solution. Staining was watched under the dissecting microscope. After three minutes the methylene blue solution was poured off and the dish containing the larva was then waved up and down for about ten seconds to expose the tissues to air after staining. The nerves became a brilliant blue and could be traced with the greatest of ease. Insect saline was then poured over the larva again for further dissection. The stain lasted for about an hour, gradually fading and becoming colorless. If the dissection was not complete, the staining procedure was repeated; the nerves again became a brilliant blue. It was found possible to repeat this procedure four times during as many hours on

one dissection with favorable results.

For studying the sclerotized parts of the head, the usual procedure of boiling the desired parts in 10 per cent caustic potash was employed. Van Gieson's stain was used to stain the unpigmented chitin for observation. The head capsule was then placed in glycerine for manipulation and dissection.

The dissections were made with a binocular dissecting microscope, using a magnification as high as 153x with extraordinary clarity. Special dissecting needles were made by dipping minuten nadlen into a mixture of nitric acid and absolute alcohol which produced very fine needles for tracing the nerves.

HISTORICAL SURVEY

Undoubtedly no other fly larvae have received as much attention as those belonging to the group Cyclorrhapha. This is readily seen because of their interesting and wide diversity of habits, as exemplified by their highly modified structure. For one finds the larvae of these flies living as parasites in animal, plant, and decaying animal matter. The blowflies belong to the last group. The literature which exists on the cyclorrhaphous fly larvae is tremendous in volume. Many times the head is merely mentioned or but briefly discussed, and to review such literature would not be justified. Therefore, the literature was carefully sifted; the review will cover only those works that have either a historical significance or a direct bearing on the problem as presented in this paper.

The earliest account of the so-called cephalo-pharyngeal apparatus that could be found was that of Leuckart (1863). His very short article consisted merely of a description of the sclerotized parts of the structure as they existed in the three larval instars of the muscids. There were no accompanying illustrations, but his descriptions left no doubt as to what he was talking about. As meaningless

HISTORICAL SURVEY

as the terms were, at least from a comparative morphological point of view, they were used for a long time afterward and some are still used at the present time.

About this same time, one of the more classical works on the embryology and post-embryological development of the muscid flies which appeared was that of Weismann (1863, 1864). Despite the fact that no histological sections were made, it contained a comprehensive description of the development of the head region. It seems that nobody ever questioned his work and to the present his conclusions concerning the development of the head of the muscid larvae are quoted as being absolutely true. As a matter of fact, no one has ever repeated his work in as comprehensive a manner. To be sure, many shorter papers have appeared on the embryology of various "acephalous" fly larvae; but none were carried through to completion, giving in detail the development of the head and its appendages.

Weismann was responsible for the idea, which still persists today, that the head of the fly larva is invaginated. Also, he concluded that the mandibles fused into a single structure which was lost after the first larval instar and was never replaced. These conclusions have led

succeeding entomologists to regard the mouth hooks as new structures and the cephalo-pharyngeal apparatus as developing on the walls of the invaginated head. Since so much of this embryological work was responsible for these viewpoints, the lines conveying these ideas will be quoted. Weismann (1863), speaking about the anterior part of the head, says:

> p. 181. "Zwischen seinem ventralen Ende und der immer noch etwas wulstigen und dicken Unterlippe befindet sich die Mundöffnung (m), und aus der gegenseitigen grösste Theil des Vorderkopfes und das ganze erste, die Mandibeln tragende Kopfsegment in die Mundspalte eingestülpt worden sein muss. Aus diesen eingestülpten Theilen bildet sich der für die Muscidenlarven so charakteristische mächtige Schlundkopf mit dem Hakenapparate."

Concerning the mandibles, he says:

> p. 184. "Demnach besitzt also nur die junge Larve wirkliche Mandibeln, die aber zu einem Stück verschmolzen sind und bei der ersten Häutung abgeworfen werden, ohne sich wieder zu erneuern."

Weismann observed the sense organs at the anterior end of the larva and described them as antennae and maxillary palps, but he considered them to be more closely associated with the thorax than the head. This viewpoint was undoubtedly arrived at through his interpretation of the invaginated head.

Brauer (1883) was the first to present an extensive account on the anatomy of the dipterous larvae. The work contained many excellend illustrations, but unfortunately, no disucssion accompanied them, as it was intended for the identification of the larvae.

An extensive piece of work on all stages of the blowfly was that of Lowne (1890 to 1892). In the first volume he presented his study of the development and morphology of the larva. His work is detailed and in many respects fairly accurate. He made histological sections of all the stages and in that way was able to make some additions and corrections to Weismann's work. However, he agreed with Weismann as to the invagination of the forehead and hence his conclusions concerning the larval head are the same. All his morphological conclusions were based on histological sections.

The investigations of Pratt (1897, 1901) on the embryology of imaginal discs in Melophagus ovinus L. were the first intensive studies on the appearance of adult structures in the egg. He showed that the cephalic imaginal discs appeared early in the embryo as single ectodermal thickenings. According to him, these cephalic buds then invaginated and, during the involution of the head, were carried forward, downward, and finally posteriorly into the new mouth along with the walls of the head. The

present paper is in agreement only with the first part of his observation, namely, that the cephalic discs are ectodermal thickenings.

Tragardl (1903), working on one of the more primitive and less specialized of the cyclorrhaphous larvae, Ephydra riparia Fall, gave a very complete anatomical picture of the cephalo-pharyngeal apparatus, identifying some of the parts and areas by the presence of sense organs. This was a definite advance over previous work, but he failed to make any dissections of the nervous system or musculature. Here again a description of the skeletal framework was made with no attempt at homologizing any of the parts.

Presumably the first attempt at explaining the reduction of the head of the dipterous larvae was that of Holmgren (1904). He took four genera of fly larvae: Chironomus, Phalacrocera, Microdon, and Musca, and studied the head of each from the point of view of comparative anatomy. Due to the fact that only four genera were used, there are many broad gaps in his presentation. However, he concluded that the mouth hooks of the cyclorrhaphous larvae were not new structures but were homologous to the mandibles of other fly larvae. No morphology of the nervous system was presented. However, in concluding his

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paper, he stated that a study of the innervation of the various parts should provide the answer concerning the various homologies.

The work of Holmgren apparently provided a great stimulus, for, beginning with Hewitt (1910), there appeared many papers in rapid succession on the mouthparts and structure of the head capsule in the dipterous larvae. Hewitt's monograph on the house fly contained a very good section on the morphology of the head region in the larva. His description included the sclerotized parts, muscles, and nerves, although the nerves were not traced completely. Many of his observations were inaccurate, as has been pointed out by subsequent workers and also verified by the writer of this paper. Hewitt intimated from morphological evidence only, that the larval head was a degenerate cephalic segment, having been permanently retracted within the body.

The same year, Becker (1910) made an extensive study of the heads of dipterous larvae to trace the homologies of the cephalo-pharyngeal apparatus and the mouth hooks. For his series he took the heads of Chironomus, Simulium, Sciara, Tipula, Pedicia, Atherix, Stratiomyia, Musca, Anthomyia, and Gastrophilus. His morphological studies

indicated that the heads of these larvae were progressively retracted into the thoracic segments and not invaginated. He also indicated that in this series of fly larvae there existed a fusion of the chitin of the pharynx with phragmata which develop from the outer head wall. He termed this structure a "Schlundkopf", composed dorsally of the head wall and ventrally of the pharynx. In respect to Musca then, he concluded that the dorsal wall of the head was actually covered by the first thoracic segment. However, with this explanation he concluded that the mouth hooks would have to be new structures and that the antennae and maxillary palps would have to be a part of the thorax. Becker ended his investigation with the suggestion that a study of the entire head should be made by means of serial sections.

Wahl (1914) made just such a study as suggested by Becker. He made serial sections of all the larval instars of Calliphora and followed the morphological nature of the head into the pupal stage. However, he failed to confirm the facts as observed by Becker. Instead, his work supported the earlier conclusions of Weismann, namely, that the larval head was invaginated and that the mouth hooks were new structures not homologous to the mandibles of other fly larvae. He further concluded that the mouth hooks were

almost solid cuticular structures with no lumen. Although Wahl's histological study was an excellent account of the anatomy of the larval head, many succeeding investigators interpreted his work as an account of head development as it takes place in Calliphora. An example of this will be noted later in this section. Wahl ended his paper by saying that another investigation of the embryology of the head region might provide the answers for the existing discrepancies.

In the various papers of Keilin (1912, 1915, 1917, 1919) was found some excellent work on the comparative morphology of the cephalo-pharyngeal apparatus of the cyclorrhaphous larvae. Apparently Keilin was not a bit concerned with invagination or retraction of the larval head. He had numerous illustrations of the sclerotized parts of the head, indicating their relationships in the various species concerned. In his discussions he gave much attention to the various sense organs found on the head, and came to the conclusion that they could be homologized to the sense organs of other dipterous larvae. His observations on the nerves going to the sense organs were extremely good and detailed, but he failed to make any further inquiry into their significance.

One of the most important works concerning the evolution of the head of dipterous larvae was that of de Meijere (1917). He presented a morphological study of a series of larval heads, starting with one which had a completely free head and ending with one in which the head was very much reduced. Throughout his investigations he homologized all the structures present and reasoned that the mouth hooks could be homologized with the maxillae of the more primitive Diptera. He concluded from his work that each structure must be considered separately since each goes ahead at a different rate in the process of evolution. From this he indicated that a definite phylogenetic series of fly larvae studied could not be set up.

Another important work on the comparative morphology of the heads of dipterous fly larvae was that of Bischoff (1922, 1924). He worked mainly on the heads of Brachycera larvae. His dissections were concerned with the musculature of the head and its appendages. He came to the conclusion that the mouth hooks of the Brachycera larvae were actually a fusion of the maxillae with the mandibles. Here, for the first time, is presented the theory that a fusion of structures might occur. He cited in one of his works (1922) an example of a cyclorrhaphous larva found in mushrooms which possessed two pairs of vertical mouth hooks. He identified these mouth hooks as the mandibles

and the maxillae. It is the only case known where such a situation has been observed and reported. Hence it has either gone unnoticed by many morphologists or was rejected as insufficient evidence.

Thompson (1920, 1928) and Miller (1932) made some excellent dissections of the head region of "acephalous" fly larvae with very accurate descriptions of the cephalo-pharyngeal apparatus and its musculature. Unfortunately, both used the accepted theory concerning the invaginated head, which makes their information useless from a comparative point of view. No mention was made of the nervous system by either of the two authors.

A very enthusiastic worker in the study of the evolution of the insects in general was Snodgrass(1924, 1926, 1928, 1935). His textbook, "Principles of Insect Morphology" (1935), presented the comparative morphology of the insects from an evolutionary standpoint and represents an outstanding piece of work. In his text he summarized the work on the dipterous larvae, including his own work on the apple maggot (1924), and drew certain conclusions concerning the heads of the muscoid larvae. Snodgrass inferred that the heads of the muscoid larvae were invaginated and that the cephalo-pharyngeal apparatus

developed on the wall of this invagination. Hence, he believed that this structure was not homologous to the head capsule of other insects. Further, he concluded that the mouth hooks are new structures developed from the neck membrane. This latter conclusion is derived from the following concepts as expressed by Snodgrass (1935):

> p. 314. "The mouth hooks of the cyclorrhaphous larva (Fig. 170 G, mhk) are often called "mandibles", but since they are solid cuticular structures, shed with each moult, arising from the lips of the atrial cavity (Atr), which is evidently derived from the infolded neck membrane, it is not clear how the larval jaws can have any relation whatever to true mandibles. Furthermore they lie in vertical planes and are moved by muscles taking their origins on the lateral walls of the cibarial pump."

It should be pointed out that Snodgrass obtained his conclusions from concepts already expressed by Weismann (1863) and Wahl (1914). It might be well to cite Snodgrass (1924) to show how he has misinterpreted the work done by Wahl. Snodgrass says:

> p.11. "The more recent paper by Wahl on the formation of the head in the cyclorrhaphous Diptera contains an exhaustive account of the head development as it takes place in Calliphora."

This statement as it exists is erroneous. The writer could find nowhere in Wahl's presentation any information as to the formation or development of the head as it takes place in <u>Calliphora</u>. Wahl was only concerned, as said earlier, with the structure of the head in the

larval instars as revealed by histological sections. Either Snodgrass did not read Wahl's account or he believed that the development of the head and the morphology of the head of a larva are synonymous.

Recently Poulson (1937, 1945) worked on the embryology of Drosophila melanogaster. In 1937 he presented a general account of the embryology in the fruit fly, and in 1945 he worked out the origin and nature of the ring gland. The account of the embryology of the fly was very sketchy and nothing of significance was given on the development of the cephalo-pharyngeal apparatus. In his account of the origin and nature of the ring gland, Poulson described the formation of the frontal sac and the chitinous bars of the cephalo-pharyngeal apparatus. His description of the cephalo-pharyngeal apparatus was so brief that it could not be considered as a presentation of its embryology. In working out the embryology of the ring gland Poulson went into great detail in describing its origin from the terminal end of the frontal sac. Since no one has identified the frontal sac morphologically, his description becomes meaningless. As will be shown in the discussion, the term frontal sac has been taken to mean different things by different investigators.

Johannsen and Butt (1941) brought together for the first time all the existing information on insect embryology. A review of the textbook revealed the state of confusion that existed among entomologists. Sometimes as many as four or five explanations were given for one phenomenon. A close examination of the book revealed that very little organogenesis was presented, particularly for the Diptera. Many times the authors did not take stand on the issues involved.

Ferris (1943, 1944, 1947) reviewed the situation of the many discrepancies that exist in the studies of the embryology and morphology of insects, especially concerning the head. He studied the external and internal morphology of the insect head from a comparative morphological point of view and found that a discrepancy existed between the neurological and morphological order of segmentation. Recognizing the fact that the nervous system of the worms and insects is organized on the same general plan, he began an evolutionary study of the nervous system as found in the head region of the Annulata. He found many errors in observations made on the nervous system of the earthworm (Lumbricus) and the clamworm (Nereis). He concluded that a reinvestigation of the entire series of Annulata should be made. This study was given over to one of his graduate students.

Henry (1947, 1948), a graduate student working with Ferris, studied the much disputed question concerning the segmentation of the insect head as revealed by the nervous system. This entailed an evolutionary study of the nervous system throughout the large group known as the Annulata, beginning with the Oligochaeta and ending with the Insecta. Henry (1948) comes to these general conclusions which are quoted here in part:

> p. 40. "It is here concluded that the stability of the nervous system may be relied upon even to very small details and that it is disturbed .....only by loss of parts, fusions, and elaborations of pre-existing parts, ... This system seems clearly to present the basic material for the determination of homologies throughout the Annulata.....It appears that a muscle is always innervated by a nerve which belongs to the segment to which the insertion of the muscle belongs. No case has yet been seen in which this arrangement is disturbed.....it appears that the innervation of muscles, at least, is a clear index to segmentation."

Undoubtedly the most significant piece of work on the morphology of the heads of dipterous fly larvae was that of Cook (1949). He presented a comparative morphological study of the heads of fly larvae, excluding the Cyclorrhapha, from an evolutionary viewpoint. Cook found that certain trends or tendencies were in operation throughout the fly larvae,and thereby indicated the course by which the heads of the cyclorrhaphous dipterous larvae

may have evolved. Cook uses the term "tendency" in the same sense as explained by Ferris (1944):

> p. 78. "An evolutionary tendency, as here meant, implies the existence of a genetic pattern and of genetic capacities which are favorable to or unstable in the direction of changes that express themselves recurrently, but perhaps irregularly, in the somatic structures of all organisms which possess that pattern and those capacities."

Some of the tendencies or patterns of evolution as suggested by Cook's study are: reduction of the head capsule into sclerotized rods; formation of phragmata; fusion of the maxillae with the mandible; rotation of mandibles; shifting of mandibular muscle origins; pharyngeal sclerotization; reduction of the labium; retraction of the head. These tendencies will be referred to again in another section of this paper when the cyclorrhaphous larval head is discussed.

DEVELOPMENTAL ANATOMY

Since this investigation on the cyclorrhaphous larval head presents ideas not in accord with the generally accepted viewpoint, it was found necessary to change the usual precedure in presenting the facts observed. The usual procedure involved in describing the morphology of the head in insects, namely, describing the external morphology first, then the musculature and innervation, did not seem appropriate. If this procedure were used, the writer would be presenting his material in reverse of the steps found necessary in obtaining his conclusions. Furthermore, the sclerotized areas of the head and the head appendages of insects in general show a multitude of variations; and the very complex cephalo-pharyngeal apparatus of the blowfly is undoubtedly the greatest modification. The musculature and the nerves, especially, have remained much more stable. Hence, if a description of the sclerotized parts of the head were taken first, it would be starting with the part least known from a comparative morphological point of view. It was decided, therefore, to present the material in an order simulating the actual procedure involved in this investigation. Embryological evidence will be brought into the morphological discussion whenever the embryology helps to confirm a morphological deduction, thereby inter-relating the two fields.

In starting the investigation, a number of embryological and morphological facts, common to all insects thus far studied and agreed on by most workers in entomology, were listed. These are sometimes referred to as the concept of landmarks, a concept rather widely used in the field of biology. The landmarks listed were::

1. The salivary glands originate as paired invaginations of the second maxillary segment (labium), their orifices fusing into a common salivary opening.

2. The common salivary duct has its opening at the base of the venter of the labium.

3. The frontal ganglion rests on the dorsal wall of the pharynx, its connectives separating the cibarial muscles (anteriorly) from the pharyngeal muscles (posteriorly).

4. The stomodaeum is an invagination of the first body segment (labrum) and gives rise to the stomodaeal nervous system.

Before considering these landmarks as they apply to the blowfly maggot, Figure 2 will be discussed in order to orient the structures involved in this problem. The salivary glands have been cut close to their common duct, as they are extremely long and wide, completely hiding from view the nervous system and structures associated with it. Occupying the greater part of the dorsal half of the body cavity in this area is the crop, an evagination or outpocketing of the oesophagus. The central nervous system with all its nerves radiating from it is located ventrally and extends from the metathoracic to about the middle of the first abdominal segment. The cephalo-pharyngeal apparatus and musculature involved is found just anterior to the crop. Huge dorsal and ventral muscles coming off its posterior end mark it well for identification. This cephalo-pharyngeal apparatus of the third instar larva, on which all the dissections are based, is approximately 1.25 mm. long, 0.33 mm. wide, and 0.50 mm. high.

The Labium and the Salivary Duct

In examining the larva from the ventral aspect, the cephalo-pharyngeal apparatus is completely hidden by broad, flat muscles which originate on the ventral wall of the metathoracic and first abdominal segments

and insert on the ventral wall of the prothorax (Figure 5B). Upon removal of these prothoracic retractor muscles, the ventral wall of the cephalopharyngeal apparatus comes into view. The common salivary duct is readily distinguished as a long, slender, chitinous tube running along the ventral wall of the apparatus in the midline. Tracing the salivary duct anteriorly, it penetrates the ventral wall of the apparatus and empties into the mouth cavity (Figure 6). This point marks the base of the labium. If this maggot has a labium, it then must be found anterior (morphologically it is posterior) to the opening of the salivary duct. In examining the area anterior to the duct opening, a series of sclerites is found. Immediately anterior to the salivary duct opening is a heavily sclerotized bar running transversely across the venter of the labial area. Anterior to this bar are found two irregularly shaped sclerites and another smaller, transverse, sclerotized bar. These sclerites just mentioned are all in the area which morphologically should be labium.

In further homologizing this area with the labium of other insects, the musculature and innervation were investigated. Two muscles are present on each side,

one of which is associated with the labial sclerites and the other with the salivary duct (Figure 5A). The labial muscles have their origin on the latero-ventral wall (the pharyngeal phragma) of the cephalo-pharyngeal apparatus and insert on the small, transverse, sclerotized bar which lies in the ventral surface of the labium just within the opening to the mouth. The apex of the labium in this area is membranous and is in the form of three lobes, a large median lobe and two smaller, lateral lobes. The labial muscles insert on apodemes of this fleshy lobe and sclerotized bar. These muscles are the labial retractor muscles. The other muscles, partially hidden by the labial retractors, are very flat and narrow. They have their origins on the latero-ventral wall of the pharyngeal phragma, somewhat anterior to the origins of the labial retractor muscles and beneath them. They insert on the posterior (morphologically anterior) surface of the salivary duct near its orifice. These muscles are found in most insects and are the salivary muscles (Snodgrass 1935). Thus we have established more evidence for the area in question as belonging to the labial segment.

Running parallel to the salivary duct on each side is a nerve which gives off branches intermittently and

finally ends in a large swelling near the apex of the labium (Figure 11). This ending is the labial sense organ. The labial nerve gives off many branches, only one of which is shown in the illustration. This branch is very short and soon bifurcates, one ramus innervating the labial retractor muscle and the other innervating the salivary muscle. Branches of this nerve not shown are those which innervate the salivary duct near its opening, the imaginal labial histoblast, and the epidermis of the labium in general. Thus the labium itself, all the structures which develop from it, and its musculature are all innervated by the same nerve. Here we see an example of segmental arrangement of the nervous system. The labial nerve, when traced posteriorly, comes off the large ventral ganglion, anterior to the prothoracic nerve and leg disc (Figure 15). More will be said concerning this nerve and central nervous system later.

Concerning the embryological development of the labium and salivary glands, an examination of Figures 16, 18, 19, 20, 21, and 23 will give the general picture. Segmentation of the anterior part of the head is quite obscure and never becomes definitely discernable. However, the lateral and ventral parts show some

segmentation for a time; and the last three head segments, mandibular, maxillary, and labial, are recognizable. It is at this time that the invaginations of the salivary glands are visible. The very early stages showing the separate invaginations of the salivary glands of the labial segment are not illustrated. However, an examination of slide material revealed that the invaginations start at about the nine hour stage. As the stomodaeum invaginates, the two ectodermal invaginations are drawn anteriorly and medially. By about the thirteen hour stage the two orifices have united medially and can be located just within the opening to the mouth (Figure 18). By the time the two invaginations meet in the midline, the labial segment has become reduced to a small median lobe. It should be noted that while the illustrations show only the common salivary duct, the two salivary glands are present and are continuing their development. The salivary duct reaches its ultimate position by the sixteenth hour of development. Figure 23 compares an embryo at this stage with a first instar larva which has just emerged from the egg. Compare these illustrations with the median longitudinal dissection of a third instar larva (Figure 6). No further changes have taken place. Figure 16, although not intended for this particular structure, traces the salivary duct in a series of drawings.

The labial sense organs develop as ectodermal thickenings of the venter of the labium just anterior to the salivary duct opening (Figure 23A). These sense organs are innervated by the labial nerves. A histological section of a first instar larva (Figure 23B) shows one of these sense organs with a fragment of the nerve attached.

The Stomodaeal Nervous System

This section of the investigation was started by a study of the egg for evidence of the development of the frontal ganglion and the remainder of the nervous system to which it belongs. The embryological work on the stomodaeal nervous system of insects indicates that it is developed from a series of invaginations of the dorsal wall of the stomodaeum. Finding the frontal ganglion in the embryo and locating its final position facilitated the morphological dissection of the cephalo-pharyngeal apparatus.

The stomodaeal nervous system in Calliphora erythrocephala takes its origin from two ectodermal thickenings on the median dorsal wall of the stomodaeum (Figure 16). These two thickenings make their appearance at about the eleventh hour stage but do not

become distinct swellings until the twelfth hour of development. The anterior thickening begins to differentiate into nervous tissue at about the fourteenth hour and then breaks away from the stomodaeal wall, migrating dorsally and slightly posteriorly. The posterior thickening seems to be somewhat advanced in its development, as it can be seen to be dividing into two masses at this stage. The anterior stomodaeal nervous tissue soon divides into two masses and these take their respective positions by the sixteenth hour of development (Figures 16 and 23A). The posterior stomodaeal nervous tissue also divides into two masses, one part remaining attached to its point of origin and the other migrating anteriorly and dorsally. Especially noteworthy is the cross section through the anterior-most stomodaeal ganglion (Figure 21) showing a pair of nerves which connect this ganglion to the central nervous system. This is the frontal ganglion and frontal ganglion connectives, respectively. What should be specially noted here is the close association of all these parts in this stage of development in the embryo. The frontal ganglion lies above the stomodaeum in the mid-line with a series of muscles anteriorly and a series posteriorly (Figure 23A). Thus is established, at least embryologically, the development of the frontal

ganglion and its connectives.

In the larva the frontal ganglion lies on the crest of a large intrinsic muscle which runs transversely from one side of the pharynx to the other. From the lateral margin of this ganglion, a nerve arises, runs laterally, and then runs posteriorly along the oesophagus. This nerve can be followed to the central nervous system where it penetrates the ventral ganglion at its junction with the cerebral ganglion (Figure 15). This establishes the nerve as the frontal ganglion connective. There is a corresponding connective on the opposite side, thus establishing the two frontal ganglion connectives which were seen developing in the embryo. The relationship of the connectives to the frontal ganglion can best be seen by referring to Figure 14. The frontal ganglion connectives divide the dilator muscles found within the cephalo-pharyngeal apparatus into two groups. The anterior group consists of two rows of muscles, sixteen in a row, which take their origins on the inner surface of the dorsal wall (cranial phragma) of the cephalo-pharyngeal apparatus and insert on the dorsal wall of the cibarium-pharynx. These muscles appear to be almost vertical in position. The posterior group consists of two rows of three muscles which also have their origins on the cranial

phragma and insert on the dorsal wall of the cibarium-pharynx. The muscles anterior to the frontal ganglion connectives, according to the concept of landmarks, thus become the cibarial muscles and insert on that part of the stomodaeum referred to as the cibarium. The muscles posterior to the connectives become the pharyngeal muscles which insert on the pharynx.

On a closer examination of the frontal ganglion, a nerve is found leaving its anterior and posterior ends respectively. The simplest and most effective way of making a dissection for the study of the anterior nerve is that of removing the entire cephalo-pharyngeal apparatus from the body and then splitting it by means of a razor blade along the mid-line from the dorsal side. One of the resulting halves retains the frontal ganglion and its anterior nerve, as illustrated in Figure 6. This nerve gives off rami to all the cibarial muscles along its path (Figure 14). This nerve is so prominent and of such length that a name seemed justified. The term "procurrent nerve " is suggested.

The nerve leaving the posterior border of the frontal ganglion passes between the two rows of pharyngeal muscles, over the small transverse muscles of

the pharynx (intrinsic pharyngeal muscles), and terminates in a ganglion on the dorsal wall of the oesophagus (Figure 13). The ganglion which rests on the dorsal wall of the oesophagus is quite firmly attached. It gives off several stout rami which innervate the muscles and wall of the pharynx, crop, and oesophagus in that area. It is the second ganglion of the stomodaeal nervous system and is derived by a division of the anterior ectodermal thickening of the stomodaeum described earlier. It is the oesophageal ganglion. A nerve arises from the posterior border of the oesophageal ganglion, goes posteriorly, passes to the right of the base of the crop, runs along the dorsal wall of the oesophagus, passes through the foramen formed by the fusion of the supra- and suboesophageal ganglia, and terminates in a ganglion on the anterior end of the proventriculus. This is the recurrent nerve common to all insects investigated.

The ganglion which is found at the posterior termination of the recurrent nerve, namely, the proventricular ganglion, is the fourth ganglion of the stomodaeal nervous system. In the discussion of the embryology, it will be recalled that the posterior ectodermal thickening of the dorsal wall of the

stomodaeum divided into two masses of cells, the posterior one of which remained attached to the stomodaeum. This is the proventricular ganglion. The anterior mass of cells from the posterior thickening moved anteriorly and dorsally during its embryological development. When the recurrent nerve is followed anteriorly from its posterior-most termination, the third ganglion is revealed. Just before the recurrent nerve passes through the cerebral foramen with the oesophagus, it gives off a dorsal branch which terminates in a ganglion adhering to the ventral wall of the aorta. This is the occipital ganglion, of which more will be said later. Figure 15 shows the four ganglia of the stomodaeal nervous system and their connections in the blowfly larva. Hence, the stomodaeal nervous system in this larva innervates those structures which develop from segment number one and the muscles which insert on segment one. This nervous system is a development of the first body segment (labral), and it is seen to be confined to the innervation of that segment. Thus, as Henry (1948) pointed out in her observations on the evolution of the nervous system in the Annulata, segmentation is best indicated by nerve centers which are segmentally arranged. The embryology of the stomodaeal nervous system and its morphological

relationships as presented here provides further evidence for Henry's conclusion.

The Labrum

The comparative morphological work on the labrum and its innervation in insects has shown it to be closely associated with the stomodaeal nervous system via the frontal ganglion connectives. In fact, in all insects, the frontal ganglion connective and the nerve to the labrum come off the tritocerebral lobe of the "brain" as a common trunk and separate, in some cases almost immediately, and in others not for some distance. This close relationship is not surprising when considered in the light of the evidence as presented by Henry (1947, 1948), that the tritocerebral ganglion is the ganglion of the first body segment, the labrum. Since the stomodaeum is an invagination of the labral segment, and gives rise to the stomodaeal nervous system, it is logical to anticipate the fact that the labral nerves tie up somewhere along the line with the stomodaeal nervous system.

The labral nerve in the blowfly larva leaves the common nerve trunk mentioned above after it enters the posterior end of the cephalo-pharyngeal apparatus (Figure 12). The labral nerve branches from this

common nerve trunk shortly before the frontal ganglion connective joins the frontal ganglion. It then proceeds anteriorly and laterally to the cibarial muscles, ending in a bulb-like swelling on the ventral wall of the labrum. This swelling is the labral sense organ. The dissection of this nerve is extremely difficult since it passes between the cibarial muscles and the heavily sclerotized paraclypeal phragma, to which it is closely applied. It is impossible to dissect this phragma without destroying the labral nerve. However, if the cephalo-pharyngeal apparatus is divided medially, as previously described, and the cibarial muscles carefully removed from one of the halves, the labral nerve can be seen intact from the frontal ganglion connective to its innervation of the labral sense organ. The labral nerve does not give off any rami along its course.

The embryology of the labrum and the labral sense organs substantiates the morphological findings. A study of Figure 16 will do more for the understanding of its development than a lengthy description. It should be particularly noted that the labrum keeps its relative position to that of the salivary duct. A fold (about which more will be said later) develops posteriorly to the labral area and eventually covers

it completely. Also, a study of Figures 17 to 21, representing successive developmental stages of the embryo, identifies the labrum by the development of the labral sense organs as ectodermal thickenings of its ventral wall. An examination of the cross-sections through the labral sense organs in Figures 17 and 21 gives quite vividly the morphological position of the labrum in the early and late embryo.

In the first instar larva, the labrum is sclerotized and presents a median tooth-like structure (Figure 23B). This is the structure which Weismann (1863) considered as the fused mandibles. This sclerotized labrum is shed at the first molt. The labrum of the second and third instar larvae is membranous.

Thus, it can be seen that the blowfly larva presents a striking similarity to other insects, at least concerning the landmarks. In the morphological landmarks considered, the muscles and innervations checked very closely with those found in other insects. Both the labial nerve and frontal ganglion connective were traced to the central nervous system and found to originate anteriorly to the prothoracic nerve and leg disc (Figure 15). It follows then, that any nerve which comes off anteriorly to the prothoracic nerve

necessarily belongs to the head. Since the nervous system of the head in the Annulata has been such a clear index to segmentation (Henry, 1947, 1948), and the nerves thus far studied in the blowfly larva have verified this fact, the nervous system will be studied further to see what it reveals concerning the remaining parts of the head.

The Central Nervous System

The central nervous system in the blowfly larva has been investigated many times before, but none of the nerves to the head region have ever been followed completely. The central nervous system is a very compact mass of tissue, showing no external sighs of segmentation. All the ganglia of the head, thoracic, and abdominal segments have fused into a single mass (Figure 15).

An examination of the ventral ganglion anterior to the prothoracic leg disc and nerve reveals the following nerves: 1. A nerve coming off at the junction of the cerebral and ventral ganglion, lateral to the oesophagus; this is the common trunk of the labral nerve and frontal ganglion connective; 2. A second nerve coming off the ventral ganglion, posterior to the first nerve; this is the labial nerve as traced earlier when the innervation of the labium

was presented; 3. A third nerve coming off the ventral ganglion dorsal to the prothoracic nerve. Since the entire nervous system is so compact, indicating a fusion of all the segmental ganglia, it is logical to assume that some of the nerves have fused also. A study of the above mentioned nerves was undertaken to see if that had happened.

The Antenna

The common trunk of the labral nerve and frontal ganglion connective, when traced from the central nervous system, divides and enters the cephalo-pharyngeal apparatus as two distinct nerves (Figure 10). The more dorsal and median nerve enters the cephalo-pharyngeal apparatus and soon divides, giving rise to the labral nerve and frontal ganglion connective respectively. These have been described previously. The other branch of the common trunk also enters the cephalo-pharyngeal apparatus, passes laterally to the cibarial muscles and then leaves the cephalo-pharyngeal apparatus in the area of the notch formed by the cranial and pharyngeal phragma (Figures 10 and 15). It runs along the surface of the mandibular abductor muscle, passing laterally to the mandible, and finally terminates as a large elliptical sensory organ. This sensory organ is associated with the dorsal conical projection at the anterior end of the larva and is the

antennal sense organ. Therefore, the first nerve coming off the central nervous system is a fusion of three nerves, the frontal ganglion connective, the labral nerve, and the antennal nerve. The common trunk of these nerves has been termed the antennal-labral nerve. The course of this nerve and its relationship to the cephalo-pharyngeal apparatus is best followed in Figure 14. There are no muscles associated with the antenna.

The embryology of the antennal sense organs can best be followed by referring to Figures 17, 18, 19, and 22. In the twelve hour embryo, when the cephalic lobe or "brain" of the nervous system is still separated from the ventral nerve chain, there appear two dorsal ectodermal thickenings, which become connected to the corresponding parts of the brain by short, stout, nerves(Figure 22A). These are the antennal sensory rudiments. These sense organs can either be the eyes or the antennae of the larva. Because of the relative positions of these organs to the imaginal sense organs of the adult (Figure 22C), it is believed that they represent the antennae. Furthermore, in practically all dipterous fly larvae, the eyes undergo reduction and in many cases are lost, whereas, the antennae are always present. As the head capsule of the embryo undergoes differential growth and reduction, the nervous system of the corresponding region begins to contract and

move posteriorly, so that at sixteen hours the antennal sensory organs are far removed from the brain (Figure 22B). At this stage of development, the cerebral ganglion have fused with the ventral nerve chain; but the antennal nerve can still be seen coming off the cerebral ganglion.

The Mandibular-Maxillary Complex

The second nerve to the head coming off the ventral ganglion has been referred to as the labial nerve in the section dealing with the labium. In following this nerve from the central nervous system, it passes anteriorly as a single nerve in close proximity to the antennal-labral nerve. However, this nerve, as previously described, does not enter the cephalo-pharyngeal apparatus. It goes along the ventral wall of the pharynx and lateral to the salivary duct (Figures 11). In the region of the pharyngeal phragma, where the labial retractor muscle has its origin, it divides into two nerves. One nerve continues anteriorly along the ventral wall of the pharynx, innervates the labial muscle, salivary muscle, and terminates in the labial sense organ. This is the labial nerve and has already been described. The other branch of this nerve disappears into the mandibular muscles (Figure 10). When these muscles are dissected, this nerve is seen to pass between

the lateral and medial heads of the adductor and abductor muscles of the mandible. A good sized ganglion of this nerve is found embedded between these muscles. From this ganglion, rami are sent out innervating all the heads of the mandibular muscles. One ramus was traced to the epidermis lining the cavity on the lateral wall of the mandible.

From the anterior border of the ganglion there arises a stout nerve which continues anteriorly between the heads of the mandibular muscles. It emerges midway between their insertions, passes laterally to the mandibles and, in close association with the antennal nerve but ventral to it, enters the bulb-like swelling at the anterior end of the larva. Here it divides into three branches. The middle branch continues anteriorly and ends in a large elliptical sensory organ. This sensory organ is associated with the ventral conical projection at the anterior end of the larva and is the maxillary palp sense organ. One of the two remaining branches goes medially and ends in a small elliptical sensory organ of the maxillary lobe. The other branch goes laterally and innervates the epidermis of the maxillary lobe. There are no muscles associated with the maxillary palps.

Thus, the second nerve coming off the central nervous system is a fusion of three nerves, the mandibular,

maxillary, and labial nerves. The common trunk of these nerves has been termed the mandibular-maxillary-labial nerve.

The mandibles are laterally compressed sclerites, lying in a vertical plane and articulating with the cephalopharyngeal apparatus (Figures 7 and 8). They have a cavity near the base on the outer surface which extends throughout the mandible. Each mandible is moved by a pair of muscles, the abductor and adductor muscles (Figure 4). The abductor muscle has two heads, which take their origins on the lateral wall of the pharyngeal phragma. It inserts on an apodeme projecting from the dorsal posterior edge of the mandible. The adductor muscle has three heads, which take their origins on the lateral wall of the pharyngeal phragma, ventral to the abductors. It inserts on an apodeme projecting from a small dentate sclerite which is continuous with the ventral posterior border of the mandible.

The embryology of the mandibular-maxillary complex is very difficult to follow and a complete picture cannot be presented at this time. However, the embryological figures clearly show that the mandibles do not fuse into a single structure, but remain separated and lie on either

side of the labrum (Figures 17 and 18). Due to the fact that the mandibles do not become heavily sclerotized until the second larval instar, identification in the late embryo is difficult.

The maxillary palp sense organs, on the other hand, are readily identified in the embryo. They develop as ectodermal thickenings just ventral to the developing antennal sense organs (Figures 17, 18, and 19). A longitudinal section through a twelve hour embryo reveals the fact that these ectodermal thickenings are connected to the ventral nerve chain before the cerebral ganglia have fused with it (Figure 22A). At sixteen hours the cerebral ganglia have fused with the ventral ganglia and they have begun to move posteriorly (Figure 22B).

Prothoracic Elements of the Head

The third nerve coming off the ventral ganglion is posterior to the mandibular-maxillary-labial nerve and dorsal to the prothoracic leg disc and nerve (Figure 15). It runs anteriorly and gives off many branches in the region of the cephalo-pharyngeal apparatus. When carefully dissected, this nerve presents a remarkable picture of segmentation (Figure 9). For one thing, it

sends branches to every muscle having its origin on the body wall and inserting somewhere on the cephalo-pharyngeal apparatus. This includes:1. the large, dorsal and ventral protractors of the head, which have their origins on the dorsal and ventral body wall respectively and insert on the posterior edge of the cephalo-pharyngeal apparatus; 2. the thin, narrow, retractors of the head which take their origin from the lateral body wall and insert on the membrane covering the anterior part of the cephalo-pharyngeal apparatus above the labrum; 3. the muscles which take their origin on the dorsal body wall and insert on a sclerotized portion between the mandibles. One branch sends rami to all the muscles of the prothoracic body wall (Figure 3A). Another branch goes to the posterior region of the cranial phragma and sends a ramus into the frontal sac. Finally, this nerve eventually joins the ventral prothoracic nerve which comes off the ventral ganglion and innervates the ventral prothoracic muscles and leg discs.

Since the first two nerves considered earlier innervated all six segments of the head, this "wandering" nerve must belong to some part of the body other than the head. As noted above, this nerve innervates the prothoracic body wall muscles and also connects with the ventral prothoracic

nerve which innervates the huge ventral retractors of the prothorax. If nerves indicate segmentation, then this nerve is also a prothoracic nerve and the muscles inserting on the cephalo-pharyngeal apparatus innervated by this nerve must be prothoracic muscles. This is the view held by the writer, and this nerve is termed the prothoracic accessory nerve.

In order to account for the prothoracic elements of the cephalo-pharyngeal apparatus, the developing embryo must be examined. At about the thirteen hour stage of development, a fold appears on the dorsal side of the embryo (Figure 16). This fold is in the shape of a U, with the open end pointed anteriorly. When studied by means of cross sections, it appears to involve only the dorsal posterior part of the head. The posterior margin is well back of the developing sensory organs and the lateral margins of the fold are medial to them. Hence, the area enclosed by this fold includes the clypeus and a part of the cranium. Since the fold starts well back of the sense organs, it is believed that it marks the beginning of the thoracic region. A study of the median longitudinal sections of embryos from the twelve hour stage through the sexteen hour stage gives an indication of what happens along the mid-line (Figure 16). In less

than four hours this fold grows over the head of the larva and completely covers it. An examination of cross sections of the embryo during this process shows that this over-growth is confined to the dorsal part of the head (Figures 17 through 21). For a while, a space can be seen between the dorsal wall of the head and the prothoracic epidermis. Later on, the prothoracic epidermis becomes closely applied to the head wall. This median over-growth of the prothorax explains the prothoracic elements of the cephalo-pharyngeal apparatus. It is conceivable that parts of this epidermis become sclerotized or at least membranous, and that muscles insert on these parts. This accounts for the innervation of these muscles by a prothoracic nerve, as described earlier.

Cephalo-pharyngeal Apparatus

This structure represents a fusion of parts of the cranium with sclerotized portions of the pharynx. The cranial phragmata represent the dorsal portion of the head of the larva which is covered by the prothoracic fold. Laterally, in the region of the clypeus, phragmata have formed which have united with the lateral walls of the pharynx. A study of the cross sections of the developing embryo reveals invaginations of the dorsal wall of the head, medial to the sensory organs. These invaginations

eventually fuse with the walls of the pharynx, resulting in the paraclypeal phragmata. The stomodaeum in this area forms a trough-like structure, its lateral walls extending upward. Anteriorly, the walls fuse with the clypeal invaginations as previously mentioned. Posteriorly, the walls extend dorsally but do not fuse with the cranial phragmata. These dorsal projections of the wall of the stomodaeum become the pharyngeal phragmata.

Cephalic Imaginal Discs

The cephalic discs associated with the ocular-antennal segment of the head are enclosed in a membranous sac, the frontal sac, (Figure 15). There are actually two sacs, one on each side of the oesophagus. The eye discs are the most posterior ones and are connected to the cerebral ganglion by means of optic stalks. No connections to the nervous system could be found for the antennal discs.

Both the optic and antennal imaginal discs make their appearances in the embryo. An examination of the cross sections show that these discs arise as ectodermal thickenings of the lateral walls of the cranium, posterior to the developing sensory organs of the larva. Also, it should be noted that these imaginal discs develop laterally to the margins of the head fold. The eye imaginal discs

become connected to the cerebral ganglia at an early stage, and a longitudinal section through a fourteen hour embryo shows that the optic stalks have already united them to the cerebral ganglion (Figure 22C). The development of the optic stalk was not observed. As the central nervous system contracts and moves out of the larval head, it takes with it the eye and antennal imaginal discs. Just how this happens was not observed but certainly the imaginal discs were not observed to invaginate through the mouth opening as reported by Pratt (1897, 1901) and as suggested by Snodgrass (1924). This will be discussed in the next section of this paper.

The labial imaginal disc was not observed in the embryo. It was first recognized in the first larval instar as a small swelling in the labial area. It is rather slow in its development, but by the third larval instar, can readily be dissected and traced to the epidermis of the labium. This imaginal disc is innervated by a branch of the labial nerve as described earlier.

The Ring Gland

This gland is located above and just posterior to the cerebral ganglion. It forms a ring around the aorta, adhering to it on the dorsal and ventral sides. The

lateral sides of the ring can be freed from the aorta. It was said earlier that the occipital ganglion of the stomodaeal nervous system adhered to the ventral wall of the aorta; this ganglion is the ventral part of the ring gland.

The dorsal part of the ring gland has a separate origin. At the time that the fold begins to grow over the larval head on the dorsal side, ectodermal cells proliferate from its posterior margin (Figure 16). These cells are in the form of a long chain and eventually extend to the region of the "brain" . The posterior-most cells of this chain are in close proximity to the aorta and come to rest on its dorsal surface. These cells later become incorporated into the ring gland.

The origins of the two lateral parts of the ring gland were not observed. However, the cells making up these parts were seen to be in close proximity to the occipital ganglion. Perhaps they are a part of it or have a separate origin from the stomodaeum. At any rate, the ring gland originates from at least two sources: from the stomodaeum and from the back part of the head.

DISCUSSION

Since this paper presents ideas not in accord with the generally accepted views of the morphology of the cyclorrhaphous larval head, it might be well to consider the various accepted conclusions as presented by Snodgrass in his text on insect morphology and compare them with results found in this investigation.

One of the conclusions found in this text is that the entire facial region of the larval head posterior to the clypeus is invaginated. In explaining the way in which this came about, Snodgrass (1935) started in by saying: "to understand the morphology of the extraordinary head structure of the cyclorrhaphous maggot, we must trace its evolution from the head structure of a simpler type, possibly from an invaginated head of the tipulid or tabanid variety."

Cook (1949) and others have reviewed the morphology of these heads and results clearly indicate that both these heads are partially retracted into the thorax and not invaginated. The result is that the head wall on the dorsal side breaks down into fragments, until in many larvae, only sclerotized bars are left. In some cases, the neck

membrane actually becomes sclerotized and acts as the supporting structure for the muscles of the head. One of the trends which Cook mentioned as operating in the evolution of the dipterous larval head is that of the reduction of the head capsule. This trend is also seen in operation in the blowfly maggot. The head capsule is reduced to two dorsal cranial fragments, and is covered by a portion of the prothorax as seen in the development of the embryo.

Snodgrass, in his assumptions, mentions a pair of lateral pouches invaginated posteriorly to the clypeus. He claims that these pouches contain the antennal and eye imaginal discs. This idea apparently came from the work of Pratt (1901) in which he describes the imaginal discs as ectodermal thickenings which invaginate from the dorsal side of the head. The two invaginations have a common opening to the outside, this opening being referred to as the frontal sac. During the involution of the larval head, according to Pratt, this common opening is drawn forward, downward, and then posteriorly through the mouth. With such an explanation it is inconceivable how the larval head could remain morphologically sound. Embryological studies, as presented in this paper, reveal no such pouches. The only structure resembling a pouch is the space formed by the overgrowth of the prothorax on the dorsal part of the

head and it has nothing in common with the imaginal discs (Figure 16).

The antennal and eye imaginal discs in the blowfly larva were found to appear as thickenings of the ectoderm in the head regions, lateral to the region of overgrowth. They are pulled back into the thorax with the retraction of the central nervous system. These discs still have a continuity with their points of origin via thin strands of tissue. These cephalic imaginal discs are no different from leg or wing discs. The latter, too, are connected to their corresponding points of origin by slender stalks, some of which are barely discernable. Because of the extreme reduction of the head capsule to mere phragmata, the future adult structures have to leave the head region. This is not new to the Cyclorrhapha; it has its beginning in the Nematocera, as for example, in Chironomus (Miall and Hammond, 1900). Here the imaginal eyes and antennae move into the prothorax for want of room to develop.

Furthermore, if the larval head is invaginated as Snodgrass indicates, how does one account for the sense organs at the anterior end of the larva? As shown in this paper, these sense organs are innervated by nerves coming off that part of the central nervous system which belongs

to the head. Embryological development indicates that they belong to the head segments.

In his discussion of the mouth hooks, Snodgrass says that they cannot possibly have any relation to the mandibles of other fly larvae. The reasons supporting his conclusions are as follows:

1. "They are solid cuticular structures, shed with each moult.

2. They arise from the lips of the atrial cavity which is evidently derived from the infolded neck membrane.

3. They lie in vertical planes and are moved by muscles taking their origins on the lateral walls of the cibarial pump."

Regarding the first conclusion, all cuticular structures are shed with each moult, including the mandibles. Close examination reveals that they are not solid structures. On the lateral basal wall there is a cavity which extends throughout the mandible. The basal part of the cavity is lined with epidermal cells.

His second conclusion is not substantiated by the embryological or morphological evidence found in this investigation. The dissections reveal that the mandibles articulate with the cephalo-pharyngeal apparatus by means of the labial sclerite. The labial sclerite, in turn,

articulates with the paraclypeal phragmata. Cook, in his studies of the dipterous larval heads, found evidence for the shifting of the mandibular articulation from the head wall to the developing phragmata or inner framework.

The third conclusion by Snodgrass concerning the vertical position of the mandibles can also be discounted. Many dipterous larval forms have mandibles which operate at a forty five degree angle to the vertical. As the head capsule breaks down and phragmata develop, the mandibular articulations shift to these phragmata, thus causing a rotation of the mandibles. Mandibular rotation is discussed by Miall and Hammond (1900) and by Cook (1949). As for the origins of the mandibular muscles on the walls of the cibarial pump (pharyngeal phragma), it has been shown that muscle origins shift whenever the area on which they normally originate is replaced. Cook found cases where only one of the mandibular muscles shifted its origin due to a reduction of the head capsule, while the other mandibular muscle retained its usual origin. In other cases, both origins shifted to phragmata developed from the head wall. In all cases the general functional position of the muscles tends to be maintained in relation to the position of the appendage operated. Hence, in the blowfly larva, where extreme head reduction has occurred, the shifting of the

mandibular muscle origins to the pharyngeal phragmata is not so anomalous as it appears.

This investigation has shown that the muscles inserting on these mouth hooks are innervated by branches of the mandibular-maxillary nerves. This brings up the question as to the exact nature of these mouth hooks. Are they mandibles, maxillae, or a fusion of the two? A study of the mandibles and maxillae in many dipterous larval forms, from a comparative anatomical point of view, has indicated that the maxillae tend to fuse with the mandibles. This trend has been extensively discussed by de Meijere (1917), Bischoff (1924), and Cook (1949). In cases where the maxilla fuses with the mandible, the resulting mandibular-maxillary complex has only three muscles inserting on it instead of four. This means that one of the mandibular or maxillary muscles has been lost. Cook has convincingly demonstrated that the maxilla fuses to the mandible and, in so doing, loses one of its muscles. He makes his deduction by a study of the muscle origins and the innervation of these muscles. In all larval forms in which a mandibular-maxillary complex is found, the mandibular and maxillary nerves have fused at their base into a common trunk. Anteriorly, this nerve branches into two, the mandibular nerve innervating the two mandibular muscles and the

maxillary nerve innervating the third muscle of the complex and the maxillary palp.

In the blowfly larva, each mouth hook is operated by two muscles, both of which are innervated by a branch of the mandibular-maxillary nerve. Another branch of this nerve innervates the maxillary palp and a membranous area surrounding it. The sclerotized portion of the mandibular-maxillary complex has been lost entirely, thus accounting for the absence of the third muscle. The only remaining parts of the maxilla are the palp and the membranous area surrounding it. It is the opinion of the writer that the mouth hooks represent the mandibles of the larva.

As further evidence supporting his idea that the mouth hooks are not mandibles, Snodgrass (1924) points out that "no cyclorrhaphous species possesses mandibles in the adult stage, and no mandible buds have been noted in the larvae". Yet, in his text on insect morphology, he admits the presence of mandibles in the tipulid larva and the absence of them in the adult. Here is a discrepancy in his logic. Miall and Hammond (1900) show the presence of mandibles in the larva of Chironomus and the absence of them in the adult. In this case, no mandibular imaginal buds are found in the larva since the adult does not possess mandibles. Hence,

the absence of mandibular buds in the cyclorrhaphous larvae does not preclude that the mouth hooks cannot be mandibles.

The trend toward sclerotization of the pharynx, as observed by Cook (1949), is also present in the blowfly maggot. With a gradual reduction of the head capsule, Cook observed that the cibarium and pharynx became sclerotized; and in Tabanus he found them sclerotized and as a single unit. Here he observed that the sclerotized cibarium-pharynx was closely adjacent to the paraclypeal phragma. In the blowfly larva the sclerotized cibarium-pharynx has fused anteriorly with the paraclypeal phragma.

As to the reduction of the labium, this trend can be observed even in the most primitive forms of the dipterous larvae. In the Nematocera, the labium has undergone reduction until very few parts remain that can be homologized with the labium of other insects. In the blowfly maggot, only a few sclerites remain. However, the salivary duct and its muscles, the muscles of the labial area, and the innervation of these muscles indicate that a portion of the labial segment exists.

Finally, due to the development of the internal cranial framework and the appearance of large imaginal

head discs, the "brain" has withdrawn into the thorax. A whole series of dipterous fly larvae could be set up showing the gradual withdrawal of the "brain" from the head capsule into the thorax subsequent to a reduction of the head capsule into phragmata.

CONCLUSIONS

From this investigation the following conclusions can be drawn regarding the morphology of the blowfly larval head when considered from the point of view of evolutionary trends, the nerves to the head, and embryological evidence:

1. The head of the blowfly larva is retracted into the thorax and is not invaginated.

2. The head capsule has been reduced to phragmata, the cranial and paraclypeal phragmata.

3. The cibarium-pharynx has become heavily sclerotized and pharyngeal phragmata have developed.

4. The dorsal, lateral, and ventral sclerotizations have fused to form the greater part of the cephalo-pharyngeal apparatus.

5. The mouth hooks are the true mandibles of the larva, and articulate with the cephalo-pharyngeal apparatus.

6. The mandibles are operated by muscles having their

origins on the lateral walls of the pharyngeal phragmata. These muscles are innervated by branches of the mandibular-maxillary nerves.

7. The dorsal sensory organ at the anterior end of the larva is the antenna and is innervated by the antennal nerve.

8. The ventral sensory organ at the anterior end of the larva is the maxillary palp and is innervated by the maxillary nerve.

9. The labium is reduced to a few sclerites and a tri-lobed membranous area. It is operated by two muscles which have their origins on the pharyngeal phragmata. The labial muscles are innervated by the labial nerves.

10. The nerves to the larval head on each side have fused into two common trunks. The anterior trunk gives rise to the frontal ganglion connective, labral nerve, and antennal nerve. The posterior trunk gives rise to the mandibular nerve, maxillary nerve, and labial nerve.

11. A thoracic fold has grown over the larval head in

the mid-line and has become sclerotized in certain areas.

12. Muscles inserting on the prothoracic elements of the cephalo-pharyngeal apparatus are innervated by a prothoracic nerve.

EXPLANATION OF ILLUSTRATIONS

The accompanying illustrations are all labeled in such a manner that they are fully self-explanatory.

The embryological drawings were all done with the camera lucida. Only those structures pertinent to the problem were included.

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ABSTRACT

The present explanation of the morphology of the head of the cyclorrhaphous fly larva appears to be out of step with the concept of evolution as revealed by the study of genetics. The generally accepted opinion is that the larval head is invaginated and the sclerotized apparatus which develops on the walls of this invagination bears no relationship to the head capsule of other insects. Furthermore, the mouth hooks possessed by this larva are claimed to be entirely new structures. Here we have new structures arising as needed by the animal without regard for any pre-existing structures. This denies the theory that evolution is an orderly process initiated by the internal environment of living material.

The present investigation was undertaken to establish the morphology of the head of the blowfly larva, Calliphora erythrocephala, and to substantiate the morphological findings with embryological evidence. This study was made with the idea that the course of evolution is epigenetic and that every structure developed had its precursor.

At the beginning of the investigation, a number of embryological and morphological facts, common to all insects thus far studied, were listed. These are sometimes referred

to as landmarks. The landmarks used were:

1. The salivary glands originate as paired invaginations of the second maxillary segment (labium). Their orifices fuse into a common salivary opening.

2. The common salivary duct has its opening at the base of the venter of the labium.

3. The frontal ganglion rests on the dorsal wall of the pharynx, its connectives separating the cibarial muscles (anteriorly) from the pharyngeal muscles (posteriorly).

4. The stomodaeum is an invagination of the first body segment (labrum) and gives rise to the stomodaeal nervous system.

An embryological study of the blowfly egg revealed the salivary glands originating as paired invaginations of the labial segment. With the reduction of the labial segment, the orifices of these invaginations met in the midline, forming a common salivary opening. This opening was drawn in with the stomodaeal invagination. A dissection of the larva revealed the salivary glands and their common duct. The opening of this duct was traced to the ventral wall of the pharynx, thus marking the base of the labial segment.

Labial and salivary duct muscles were found, all innervated by branches of the labial nerve.

The stomodaeal nervous system was found to originate in the egg as two dorsal, ectodermal thickenings of the stomodaeum, each of which divided into two, forming the four stomodaeal ganglia of the nervous system. In the larva, these four ganglia were located and found to be connected by the recurrent nerve. The anterior-most ganglion, the frontal ganglion, was found on the dorsal wall of the pharynx, its connectives to the central nervous system separating the cibarial muscles from the pharyngeal muscles. The labral nerves, which in all insects unite with the frontal ganglion connectives, were found to do the same in this maggot. Tracing the labral nerves anteriorly identified the labral area. An embryological investigation substantiated the labral area of the larva.

With the landmarks thus established, a study was made of the nerves leaving the central nervous system and going to the head region.

Three nerves were found leaving the central nervous system anterior to the prothoracic leg disc and nerve. The first or most anterior of these came off at the junction

of the cerebral and ventral ganglia. This nerve divided into two branches in the region of the cephalo-pharyngeal apparatus. One branch went medially and connected with the frontal ganglion and labral sense organ. The other branch continued anteriorly and innervated the antennal sense organ. Thus, the anterior-most nerve of the central nervous system was found to be a fusion of three nerves: the frontal ganglion connective, the labral nerve, and the antennal nerve. It was called the antennal-labral nerve. An embryological study showed this sense organ as an ectodermal derivitive of the head, and its innervation with the cerebral ganglion identified it as a sense organ belonging to the third head segment. It was concluded that it represented the antenna of the larva.

The second nerve came off the central nervous system posteriorly to the antennal-labral nerve. It passed anteriorly along the ventral wall of the oesophagus. In the region of the cephalo-pharyngeal apparatus it divided into two branches. One branch continued anteriorly and innervated all the structures and muscles which belonged morphologically to the labium. The other branch proceeded dorsally and anteriorally. It innervated the mandibular muscles and the maxillary palp sense organ and lobe. Hence, the second nerve of the central nervous system was found to be a

fusion of three nerves, the mandibular, maxillary, and labial nerves. It was called the mandibular-maxillary-labial nerve. An embryological study of the head revealed that the maxillary palp sense organ developed as an ectodermal thickening of the head and established its nerve connection to the ventral ganglion.

The third nerve came off the central nervous system posteriorly to the mandibular-maxillary-labial nerve and dorsal to the prothoracic nerve. It proceeded anteriorly and innervated the muscles of the prothoracic body wall, the dorsal and ventral protractors of the head, the retractors of the head, and then joined the prothoracic nerve ventrally. It was termed the prothoracic accessory nerve. An embryological study of the developing embryo revealed a thoracic overgrowth of the larval head in the mid-line. This accounted for the prothoracic innervation of the muscles inserting on the cephalo-pharyngeal apparatus.

The cephalo-pharyngeal apparatus is represented by two cranial phragmata located dorsally and two paraclypeal phragmata located laterally. These phragmata fused with the heavily sclerotized cibarium-pharynx ventrally. The lateral walls of the pharynx developed phragmata which extended dorsally and became the attachment for the

mandibular muscles. Embryological evidence substantiated these conclusions.

The antennal and eye imaginal discs appeared early in the developing embryo as ectodermal thickenings of the head wall, posterior to the developing larval sense organs and lateral to the prothoracic head fold. The eye imaginal disc became attached to the cerebral ganglion by the optic stalk and during the retraction of the nervous system and reduction of the head capsule, was pulled posteriorly into the thorax.

The conclusions presented as a result of this investigation were:

1. The head of the blowfly larva is retracted into the thorax and is not invaginated.

2. The head capsule has been reduced to phragmata, the cranial and paraclypeal phragmata.

3. The cibarium-pharynx has become heavily sclerotized and pharyngeal phragmata have developed.

4. The dorsal, lateral, and ventral sclerotizations

have fused to form the greater part of the cephalo-pharyngeal apparatus.

5. The mouth hooks are the true mandibles of the larva, and articulate with the cephalo-pharyngeal apparatus.

6. The mandibles are operated by muscles having their origins on the lateral walls of the pharyngeal phragmata. These muscles are innervated by branches of the mandibular-maxillary nerves.

7. The dorsal sensory organ at the anterior end of the larva is the antenna and is innervated by the antennal nerve.

8. The ventral sensory organ at the anterior end of the larva is the maxillary palp and is innervated by the maxillary nerve.

9. The labium is reduced to a few sclerites and a tri-lobed membranous area. It is operated by two muscles, which have their origins on the pharyngeal phragmata. The labial muscles are innervated by the labial nerves.

10. The nerves to the larval head on each side have fused into two common trunks. The anterior trunk gives rise to the frontal ganglion connective, labral nerve, and antennal nerve. The posterior trunk gives rise to the mandibular nerve, maxillary nerve, and labial nerve.

11. A thoracic fold has grown over the larval head in the mid-line and has become sclerotized in certain areas.

12. Muscles inserting on the prothoracic elements of the cephalo-pharyngeal apparatus are innervated by a prothoracic nerve.

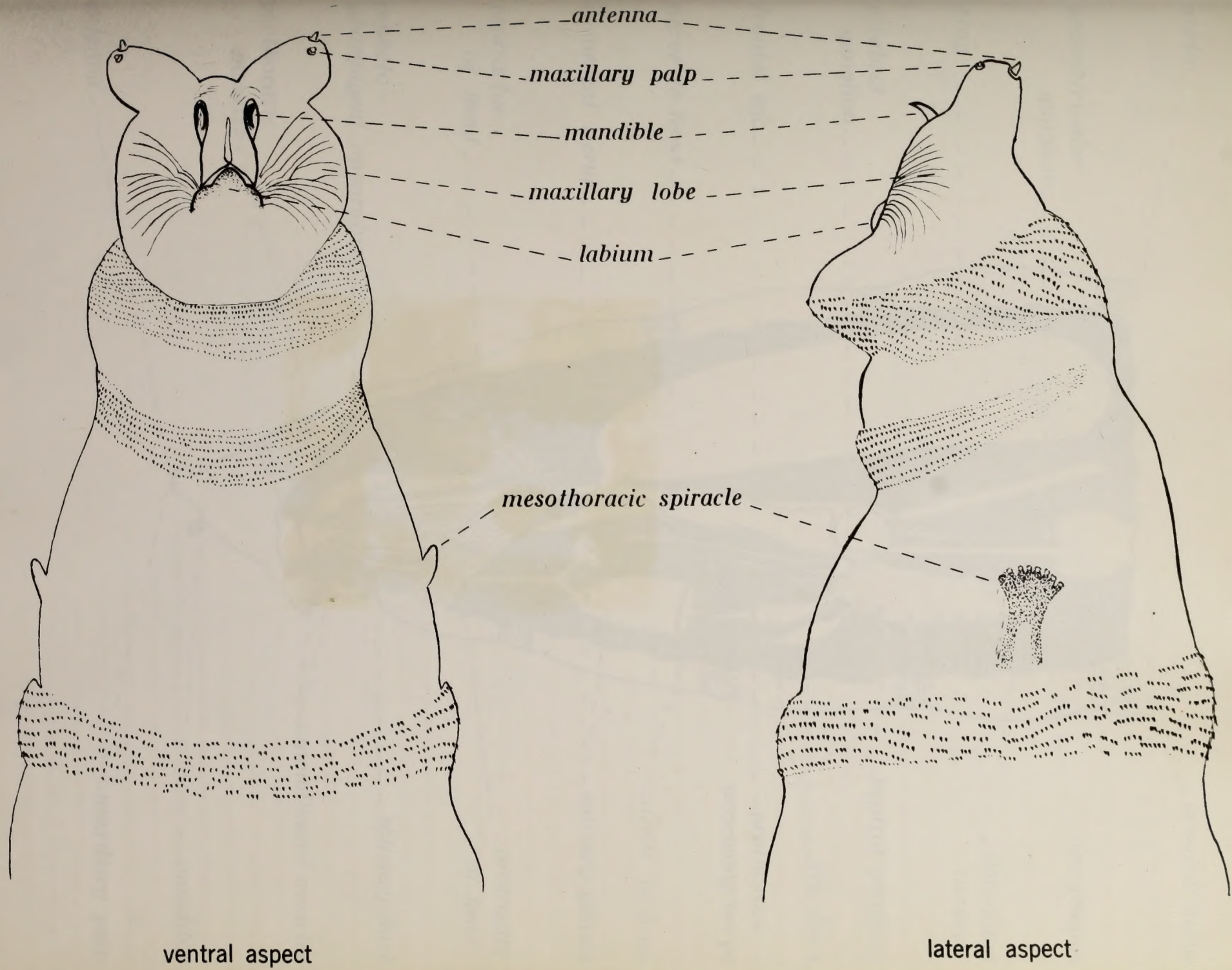
AUTOBIOGRAPHY

Carl Edward Ludwig was born September 4, 1915, in Manchester, New Hampshire. His parents were Reinhold and Selma Ludwig. He attended grammar school and the first year of high school in Manchester. Then in 1928 the family moved to Fitchburg, Massachusetts, where Ludwig graduated from high school in 1931.

The candidate majored in mathematics and science at Fitchburg State Teachers College, where he received his B.S.E. degree in 1935. From there he went to Boston University to do graduate work in biology, and earned his A.M. degree in 1936.

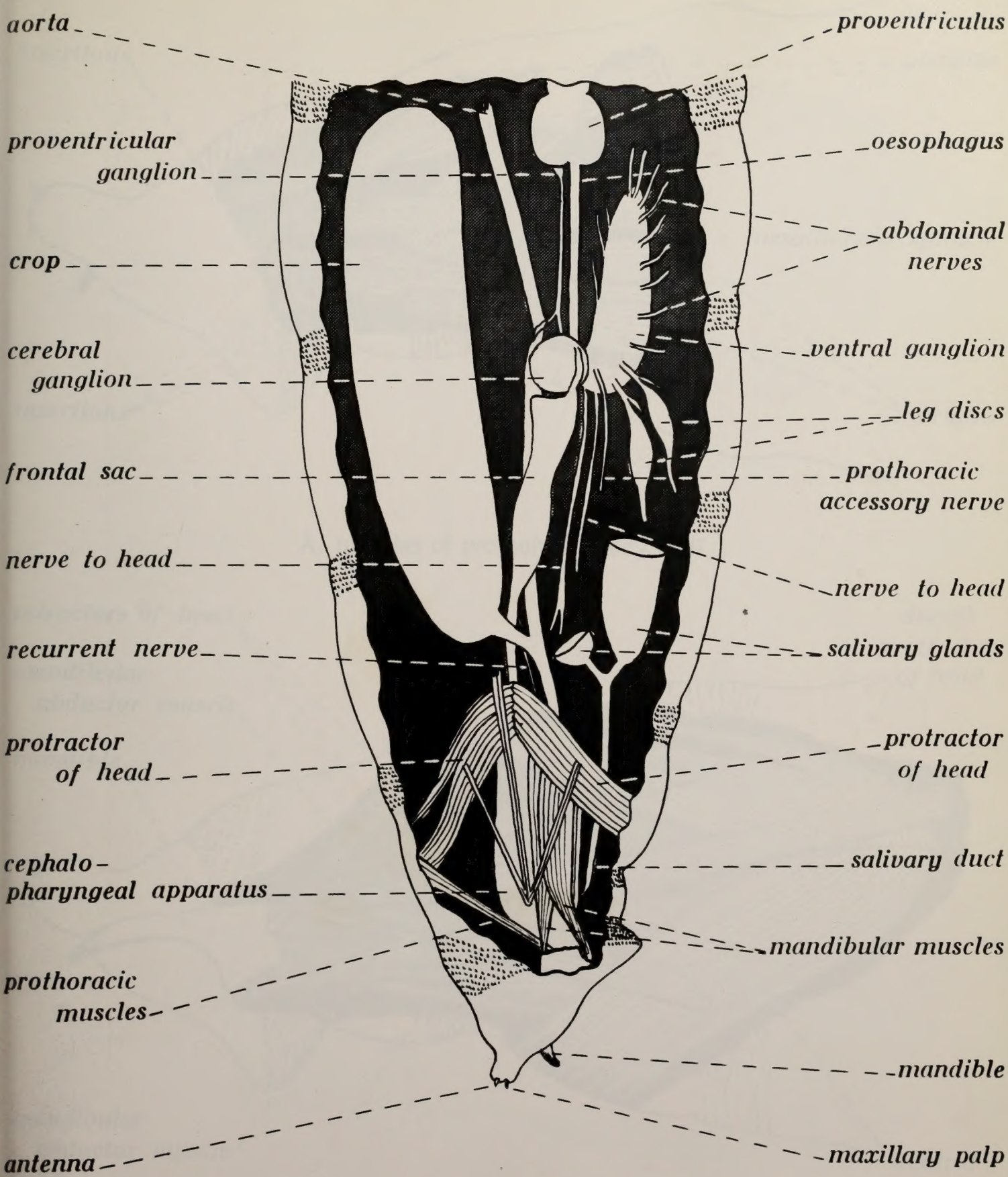
From 1936 to 1941 Ludwig had teaching fellowships at Boston University, where he also took graduate courses with the idea of working toward a Ph.D. degree. He spent two summers studying at Cornell University, New York. He then became an instructor at Boston University until 1942, when he voluntarily enlisted as an officer in the United States Naval Reserve. Ludwig served as an anti-submarine warfare instructor during the first years of the war, and as a malariologist during the last year.

After his discharge from active duty, Ludwig returned to his post as instructor at Boston University from 1946 to 1947. He then registered at Stanford University in order to carry on research. It was there that the problem for his dissertation was completed.



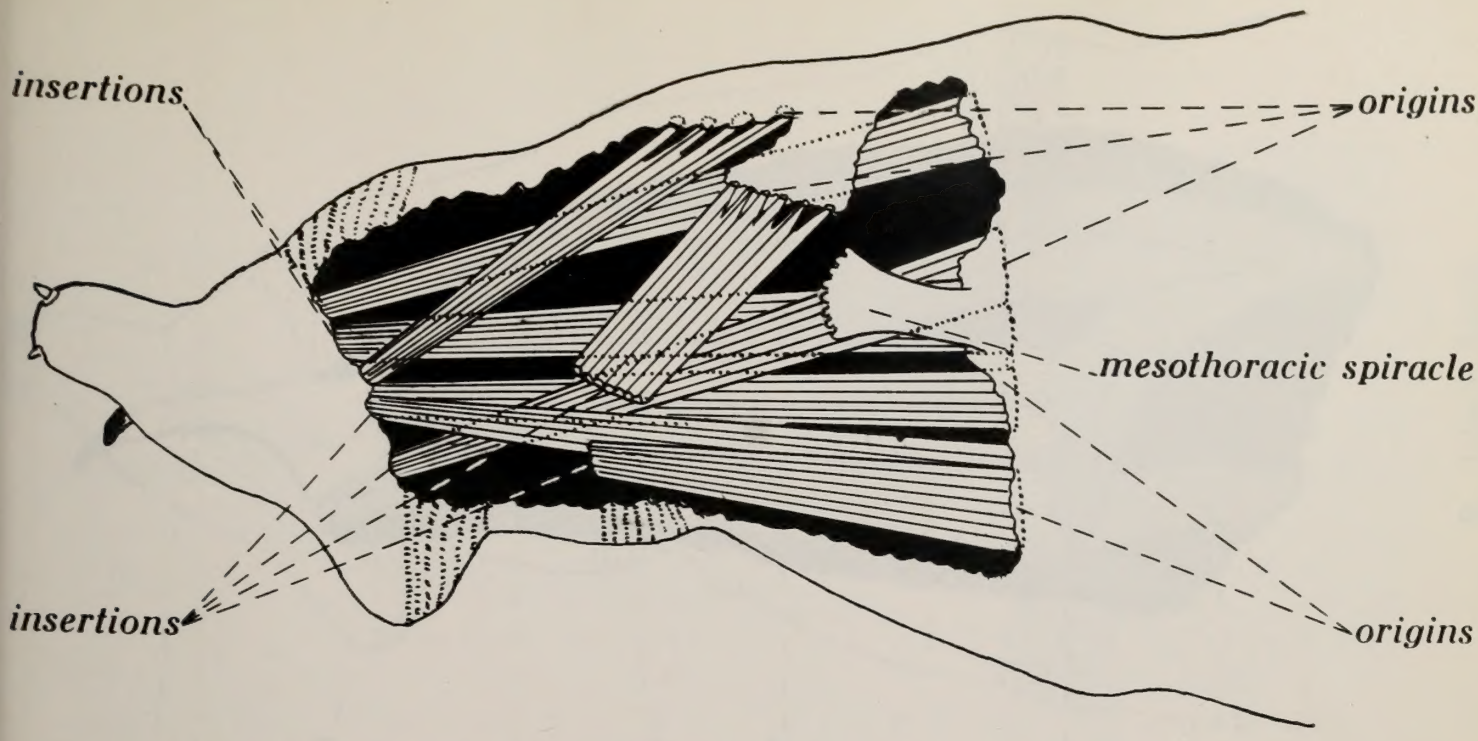
Calliphora erythrocephala Meigen ,head of larva ,external morphology

Figure 1

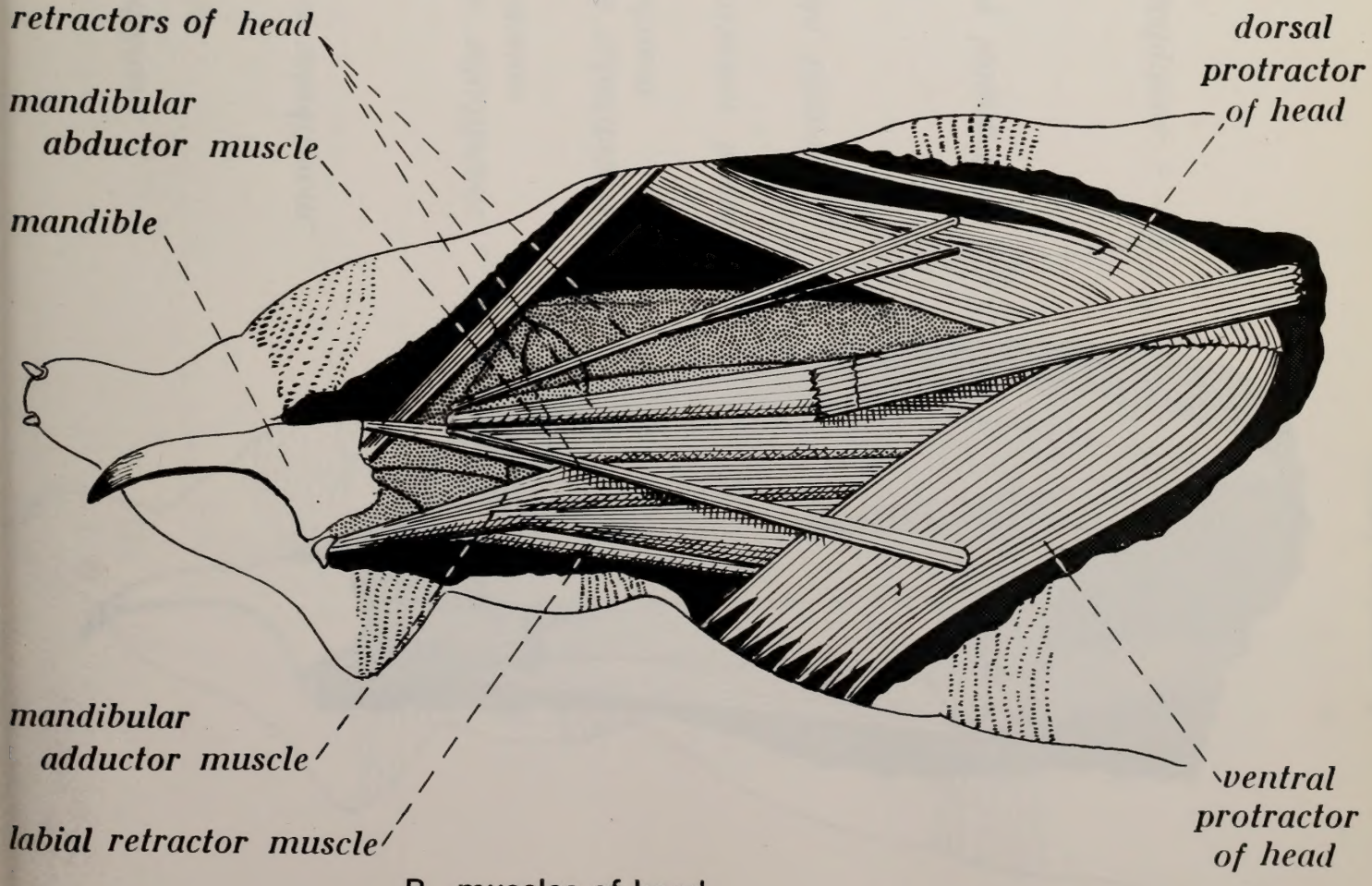


Internal morphology of larva, lateral aspect, semidiagrammatic

Figure 2



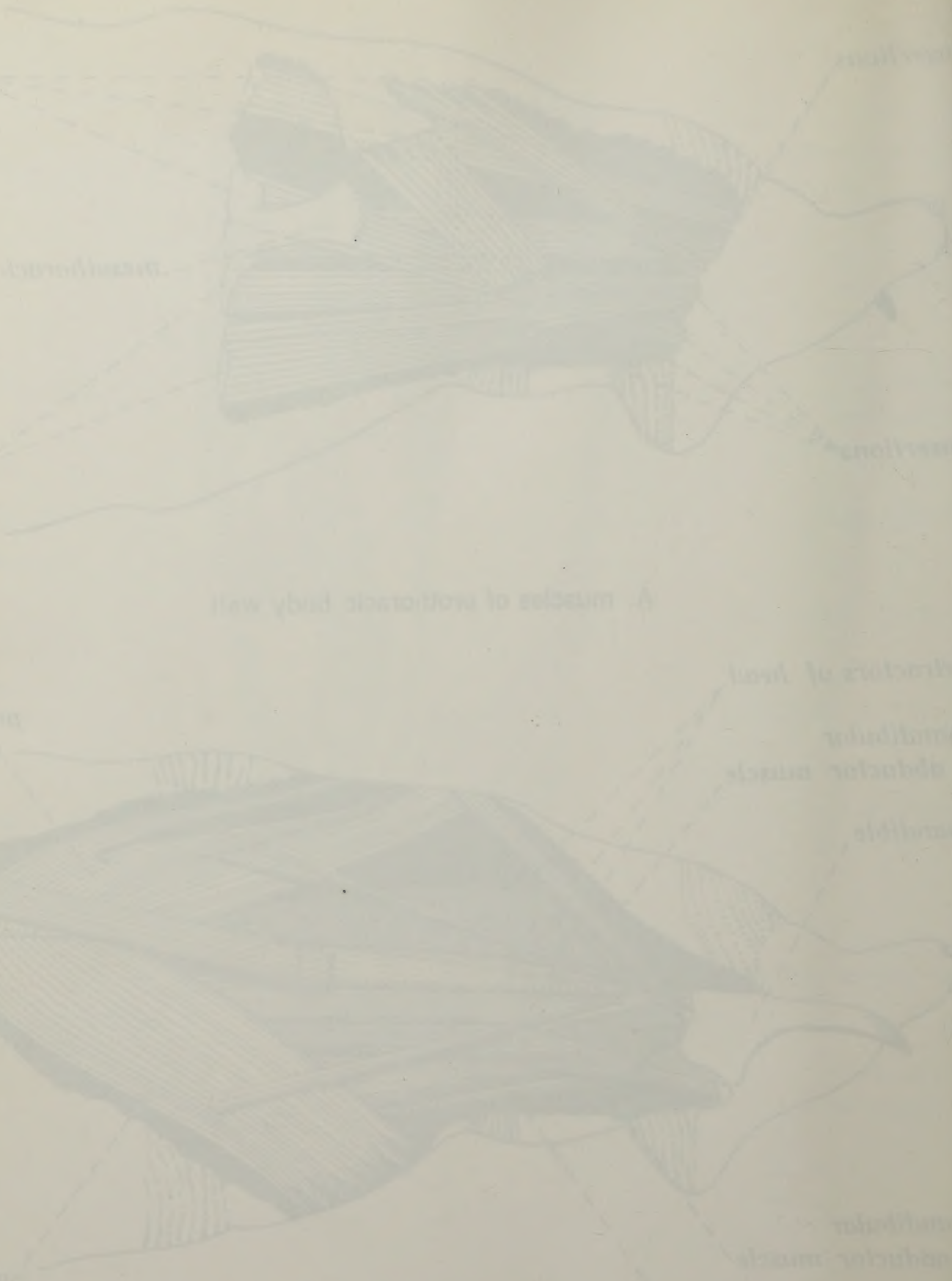
A. muscles of prothoracic body wall



B. muscles of head

Relation of musculature to external morphology, lateral aspect

Figure 3



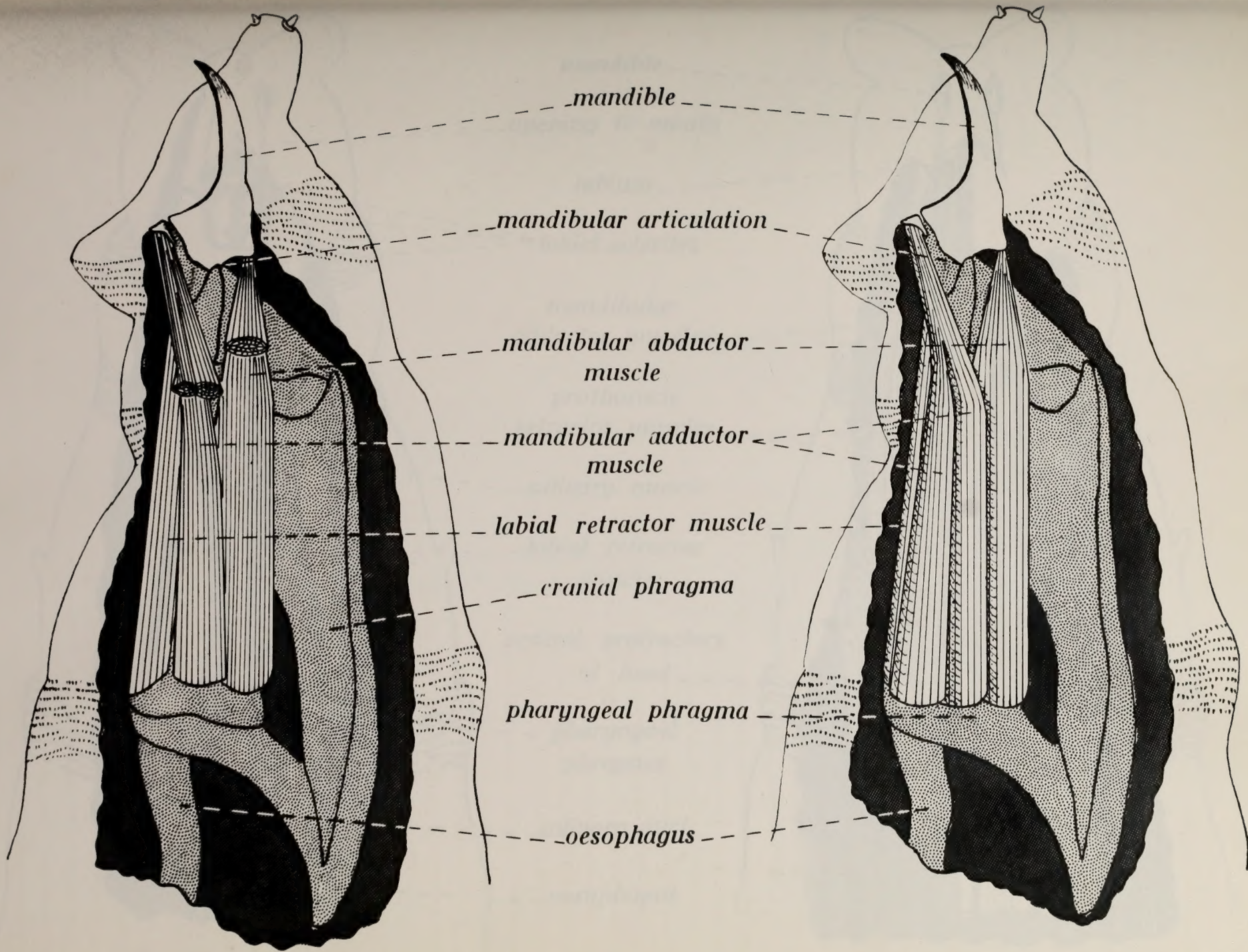
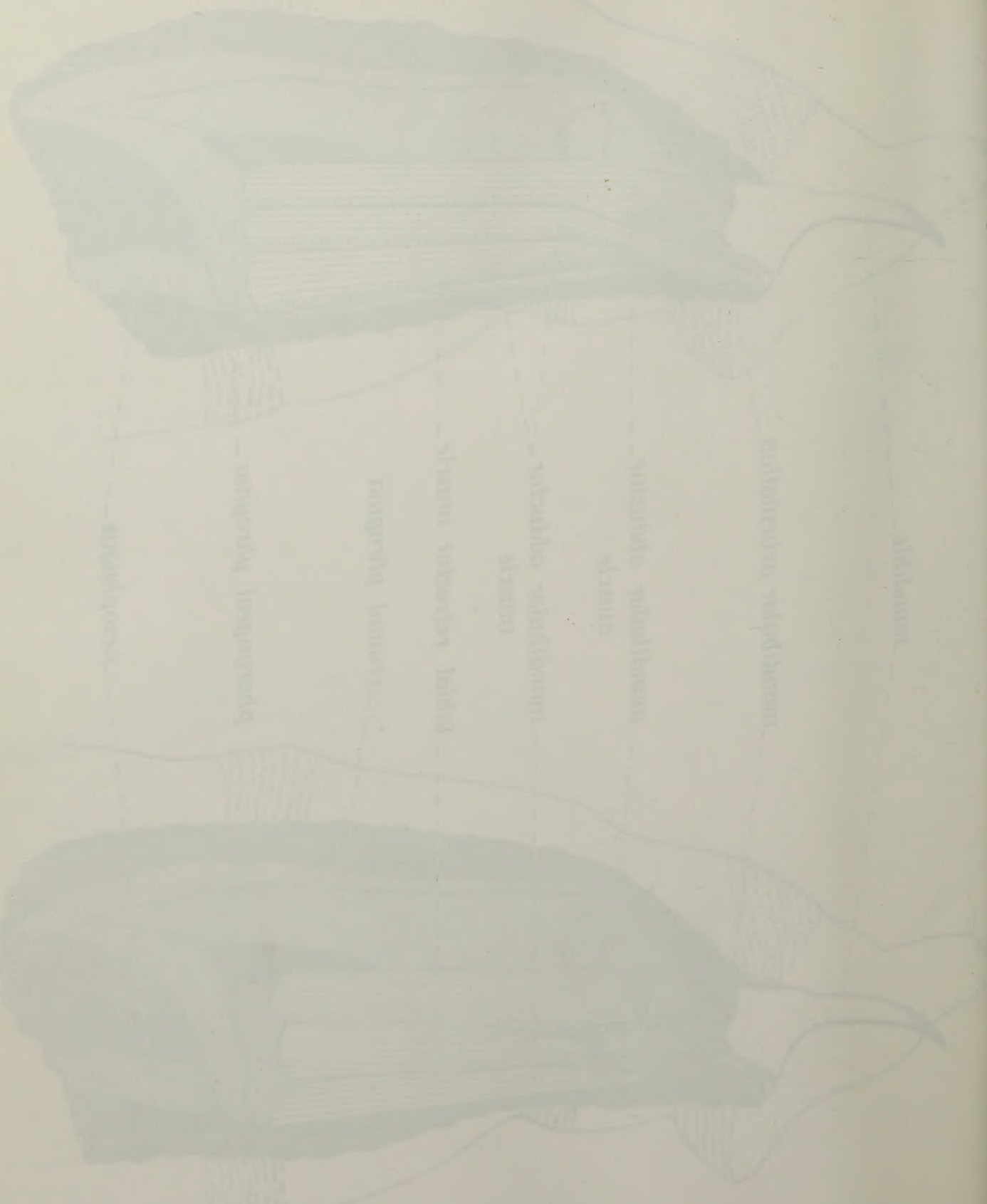
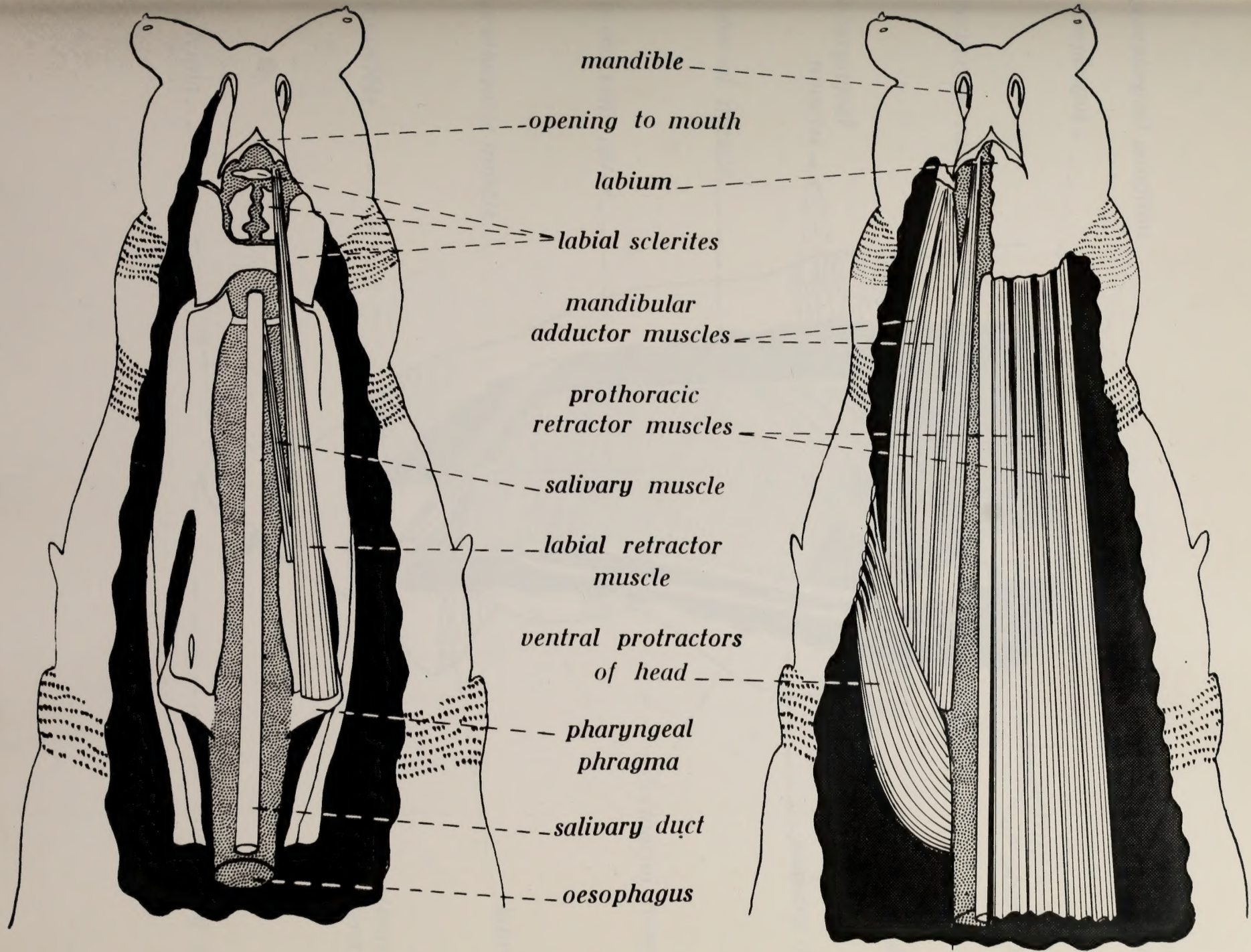


Figure 4 Musculature of mandible, lateral aspect



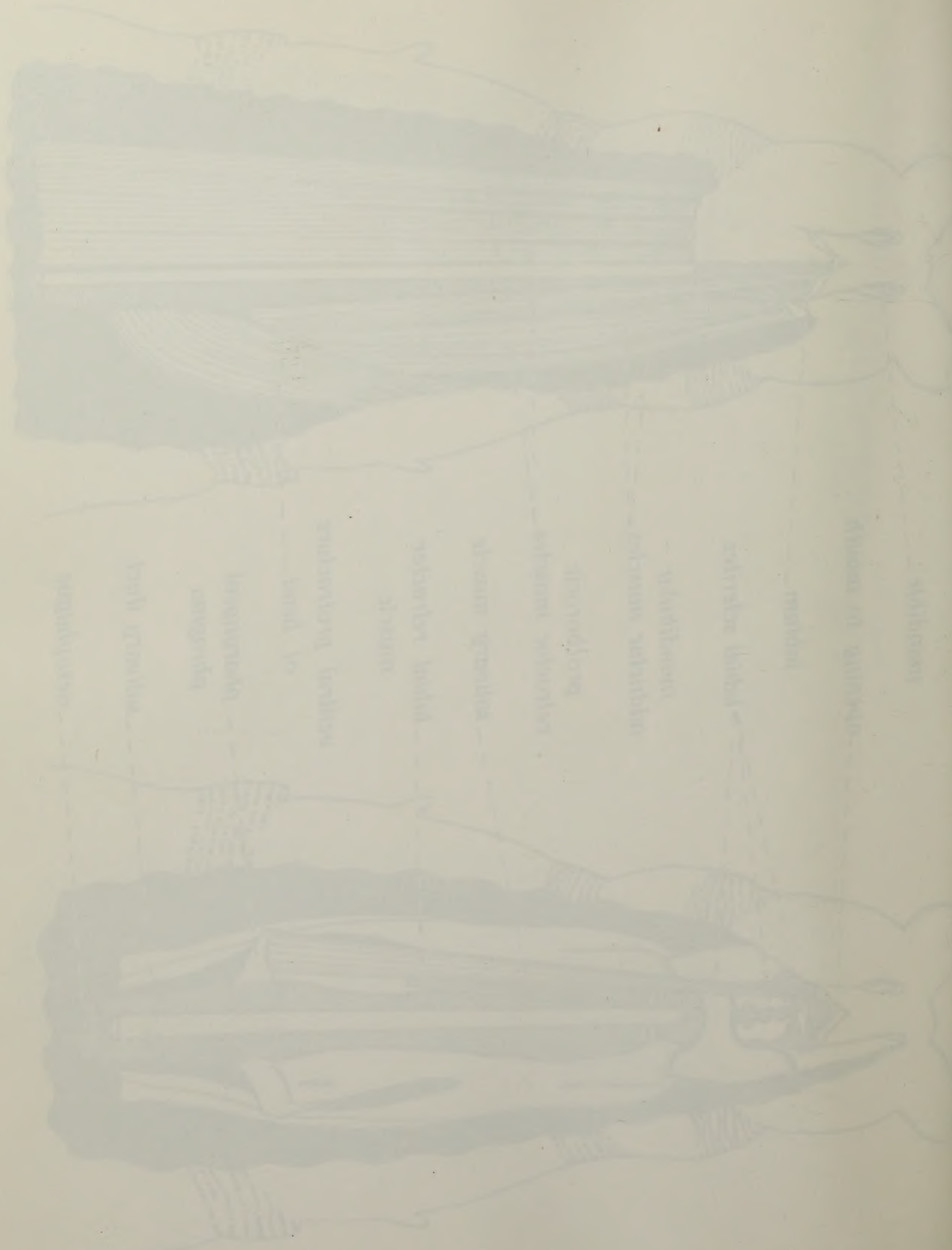
Musculature of head, ventral aspect

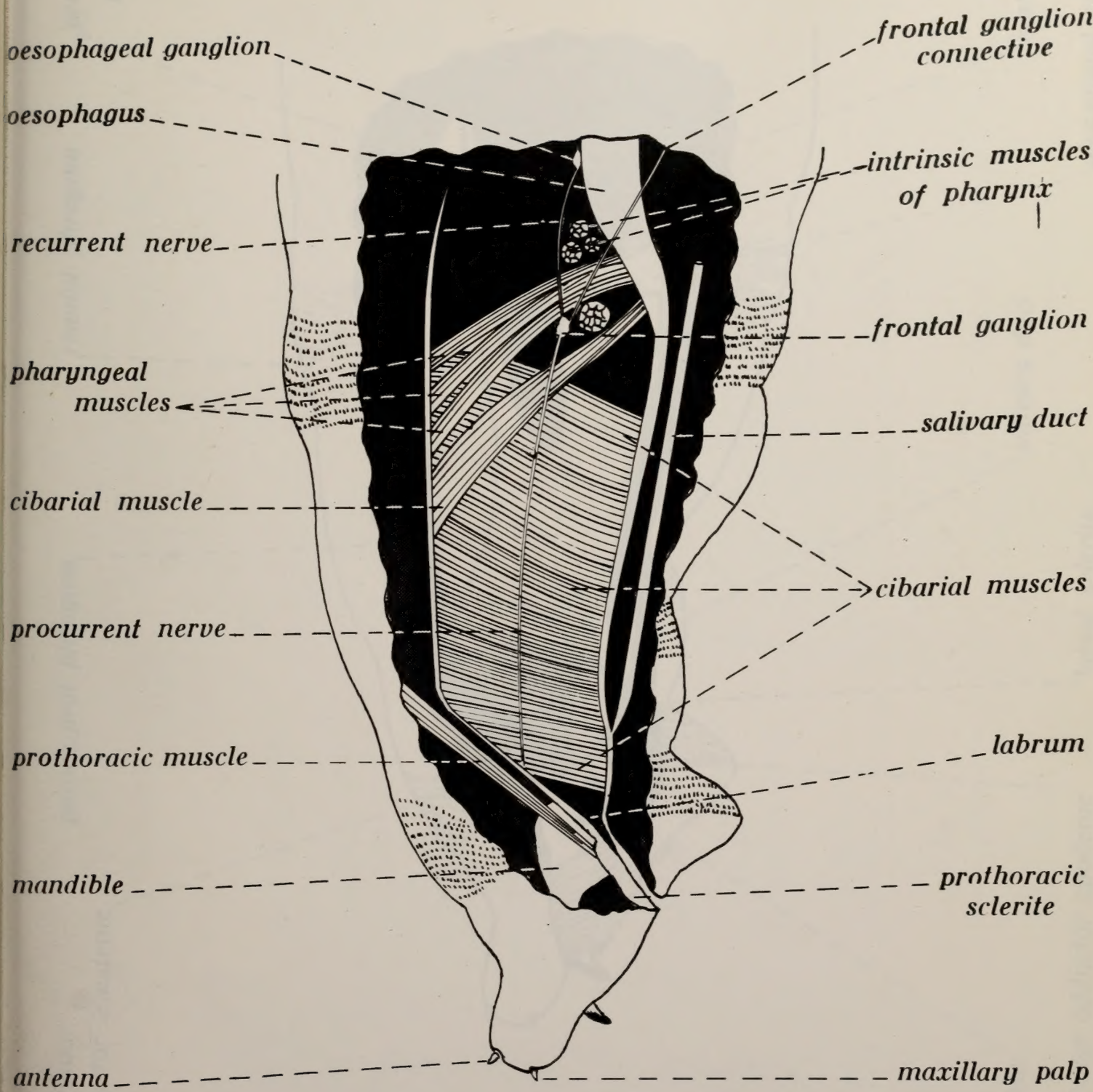


A. muscles of labium

B. muscles of mandible and prothorax

Figure 5





Musculature of cibarium–pharynx, lateral aspect

Figure 6

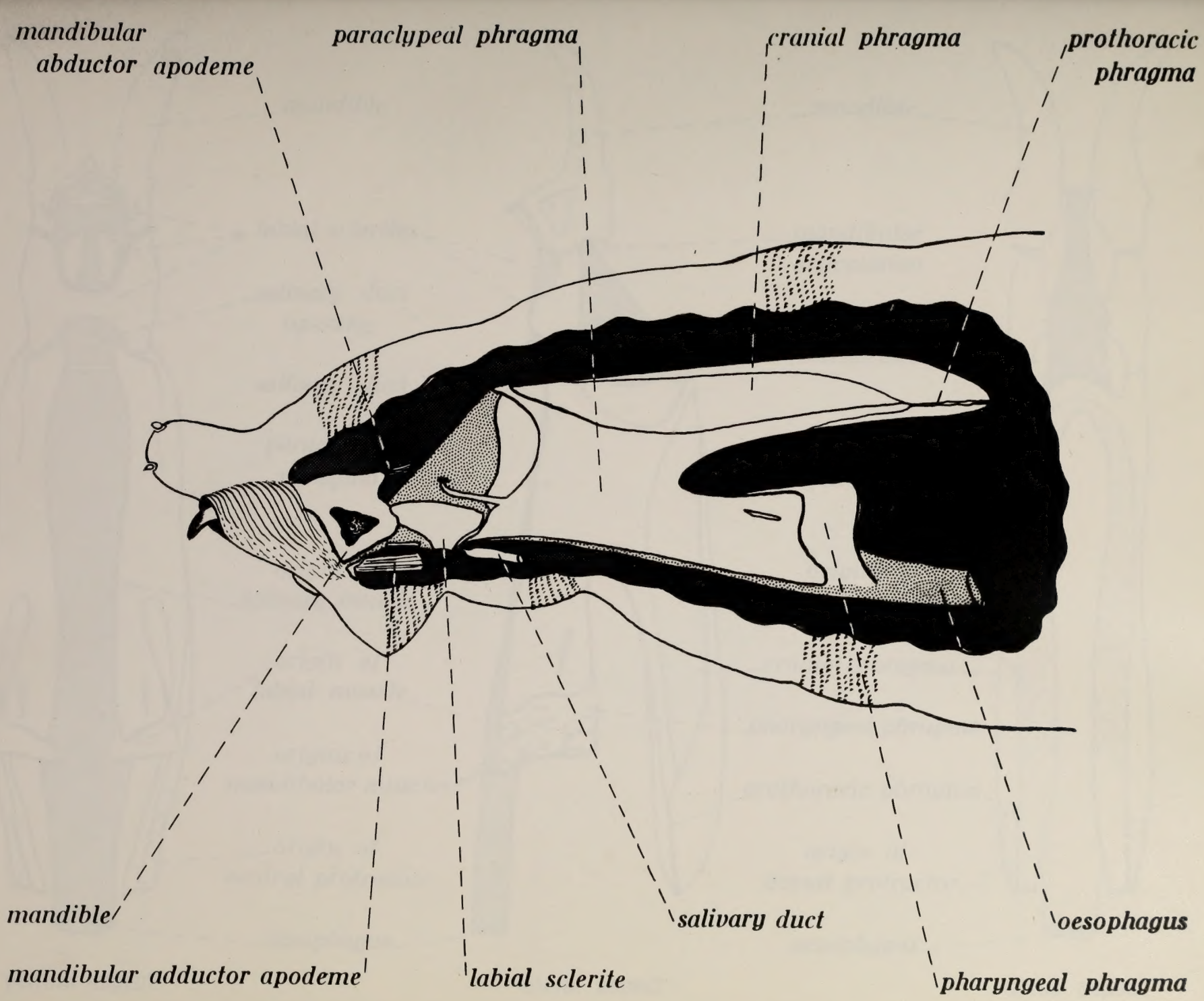
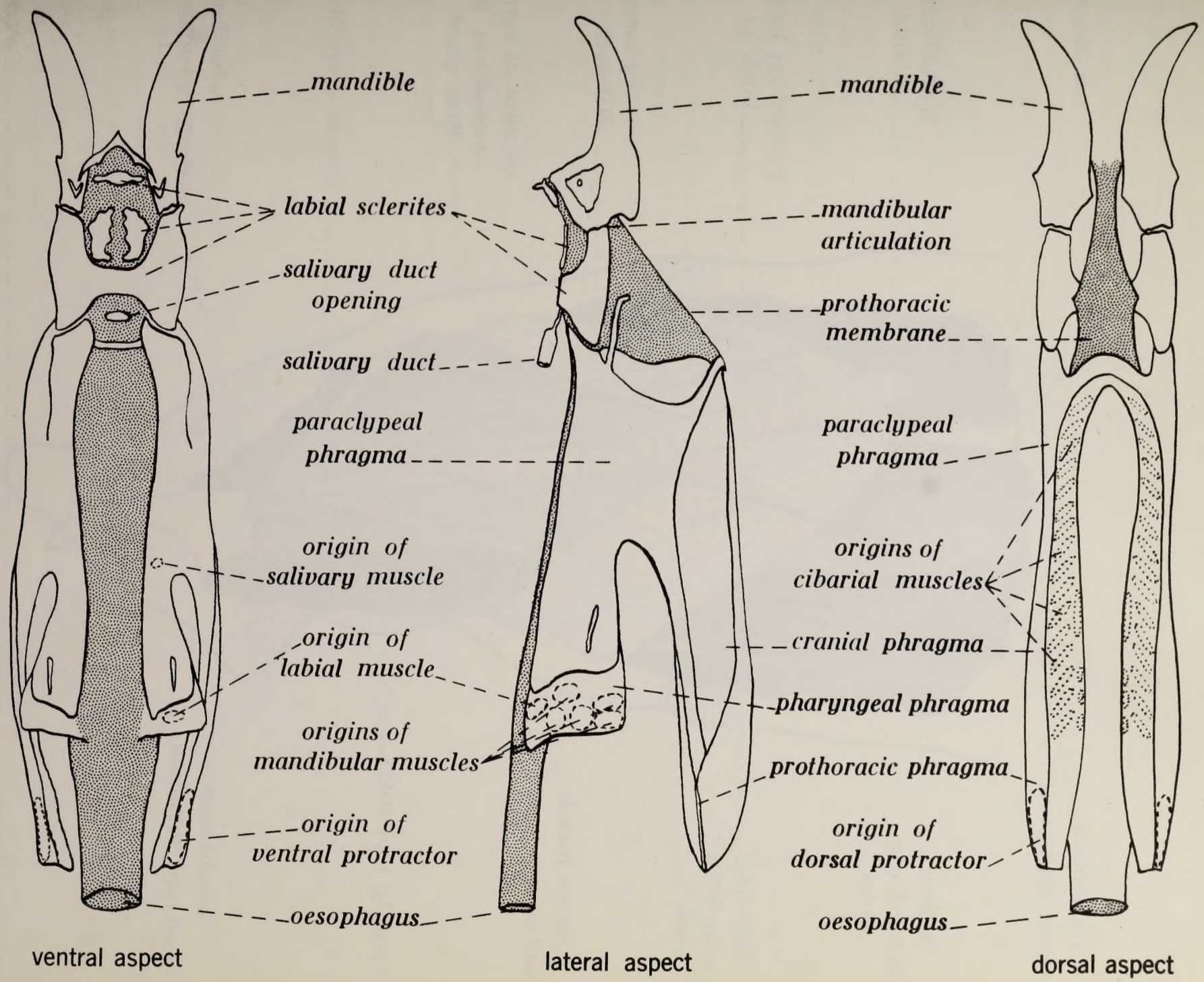
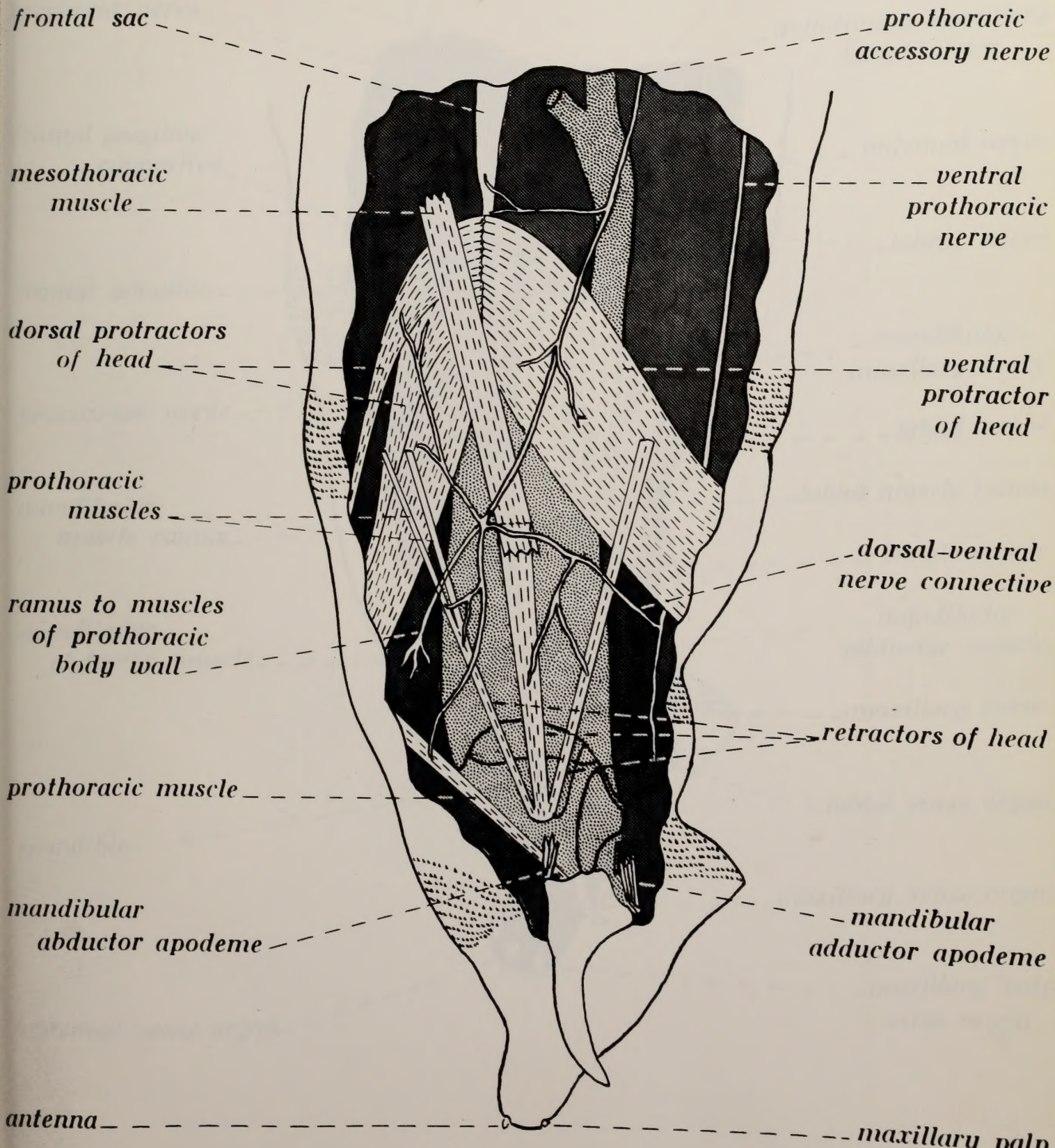


Figure 7 Cephalo-pharyngeal apparatus in position, lateral aspect



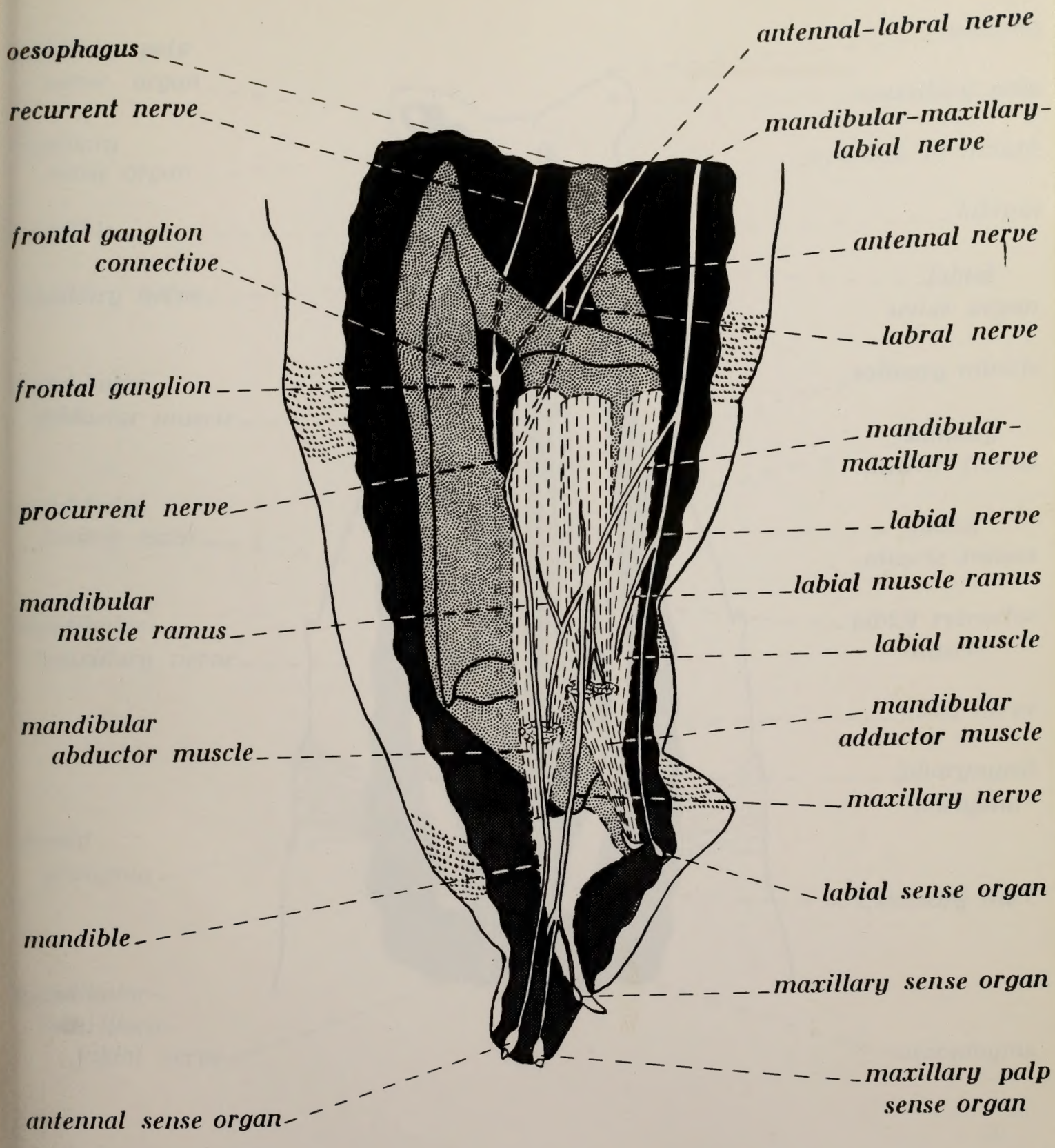
Cephalo-pharyngeal apparatus

Figure 8



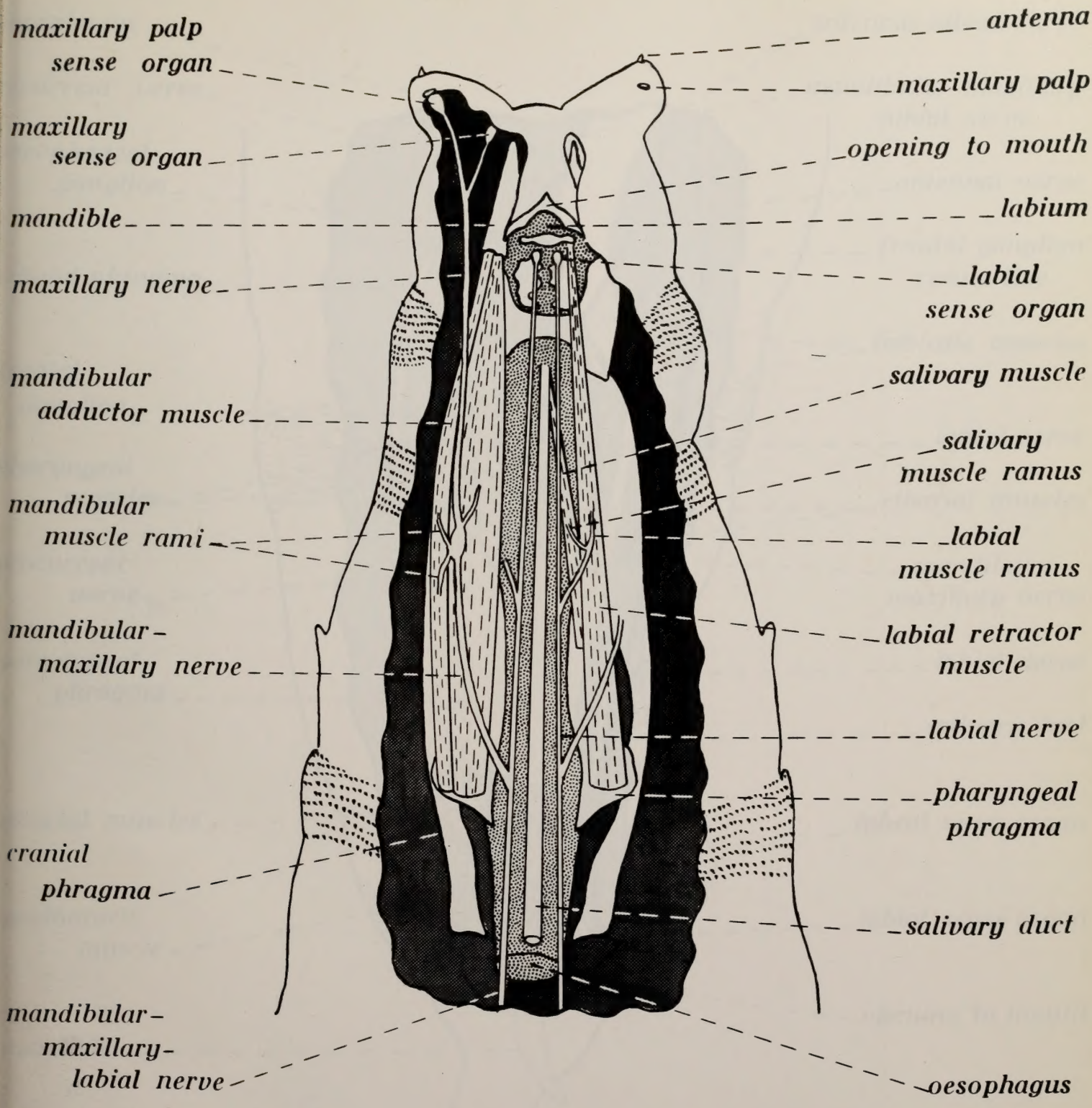
Prothoracic accessory nerve, lateral aspect

Figure 9



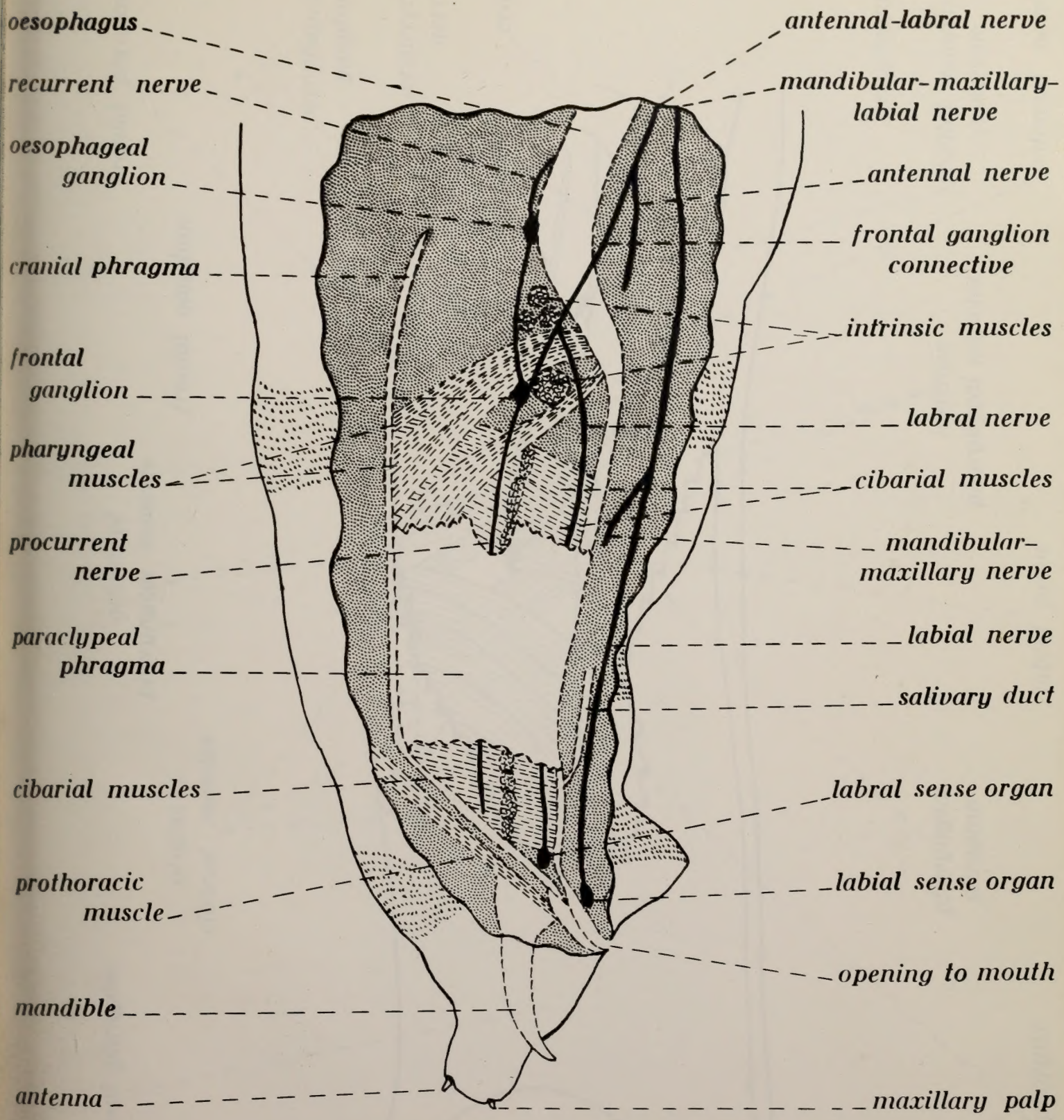
Nerves to mandibular–maxillary complex, lateral aspect

Figure 10



Nerves to mandibular–maxillary complex and labium, ventral aspect

Figure 11



Stomodaeal nervous system and labral nerve, lateral aspect

Figure 12

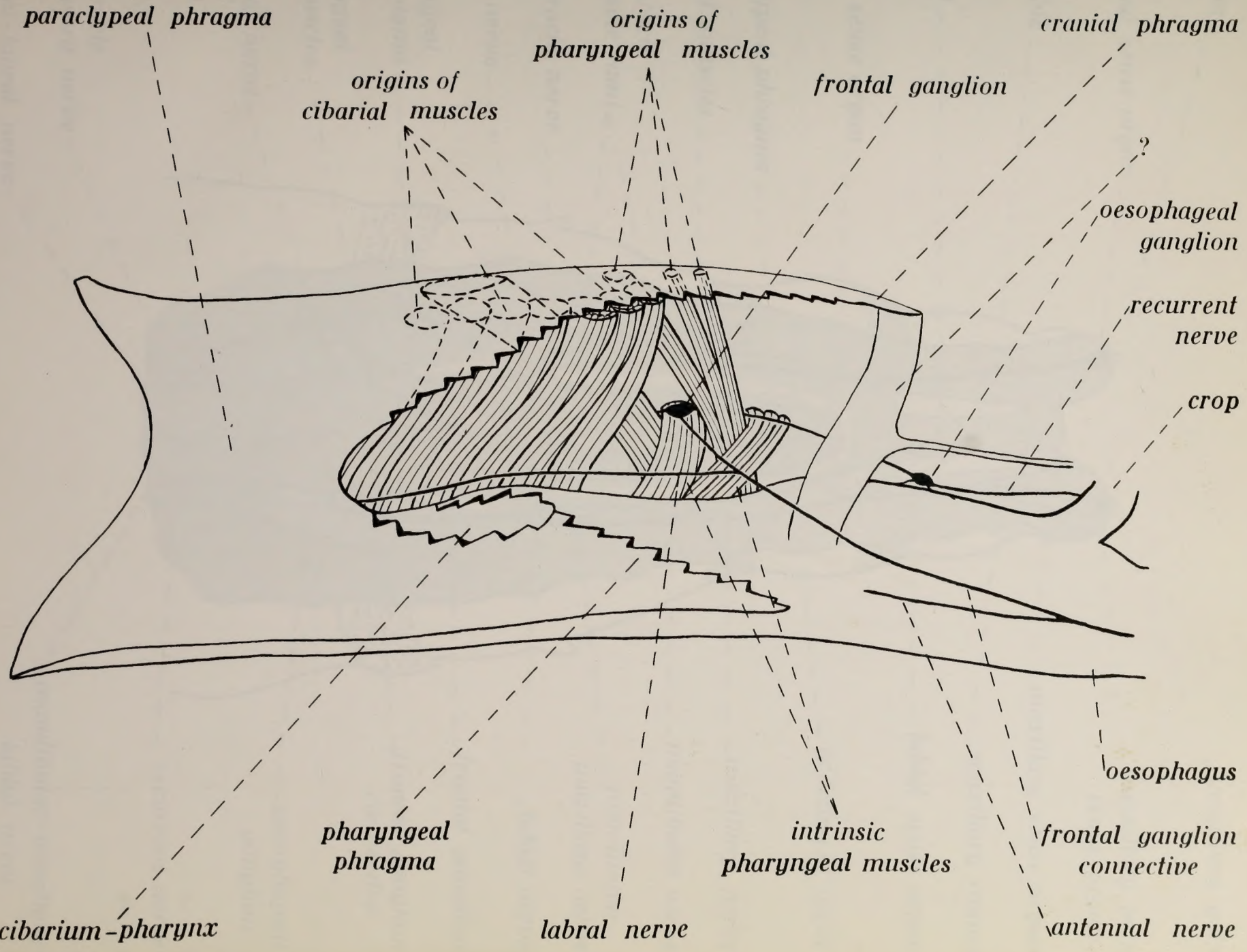
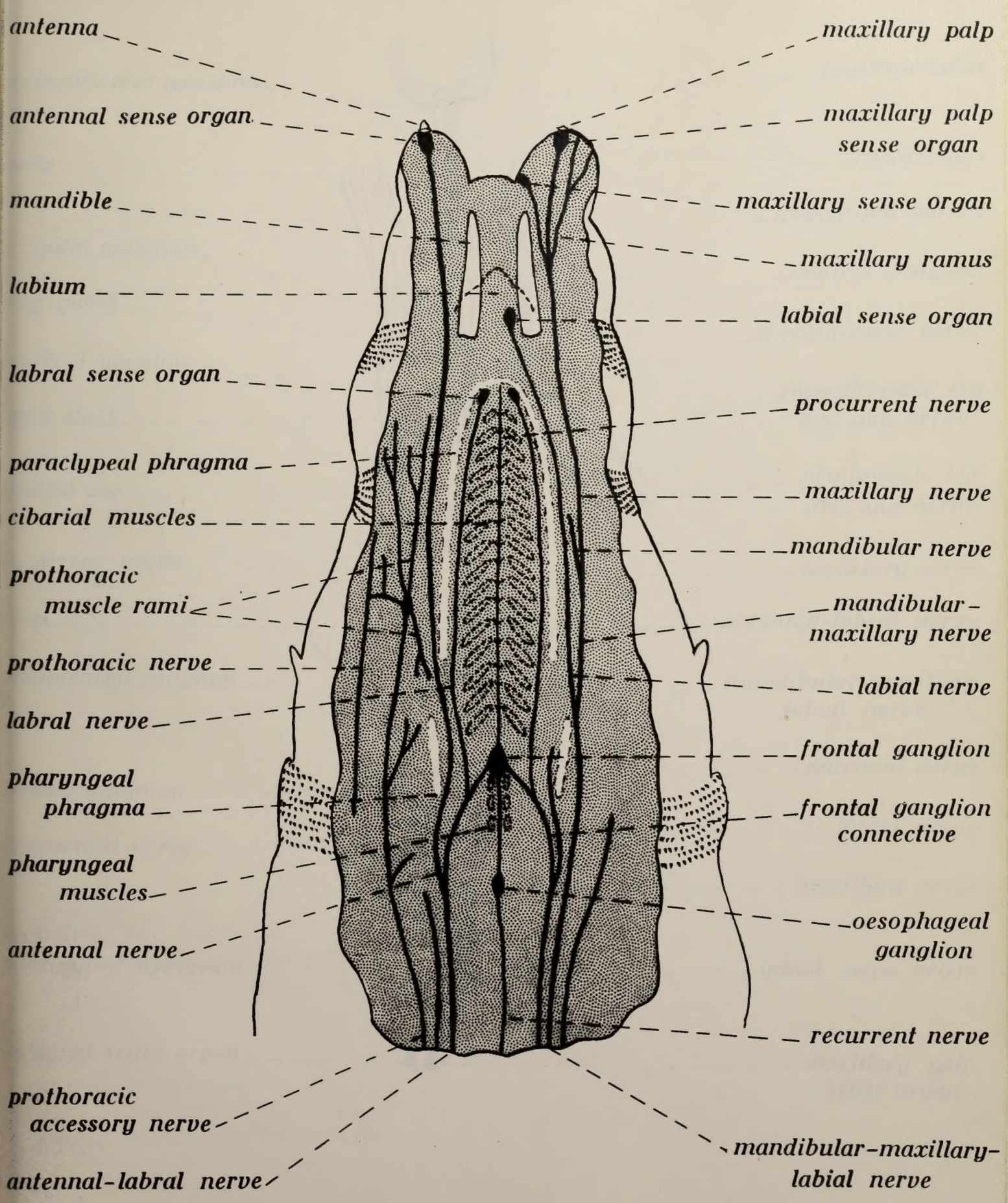
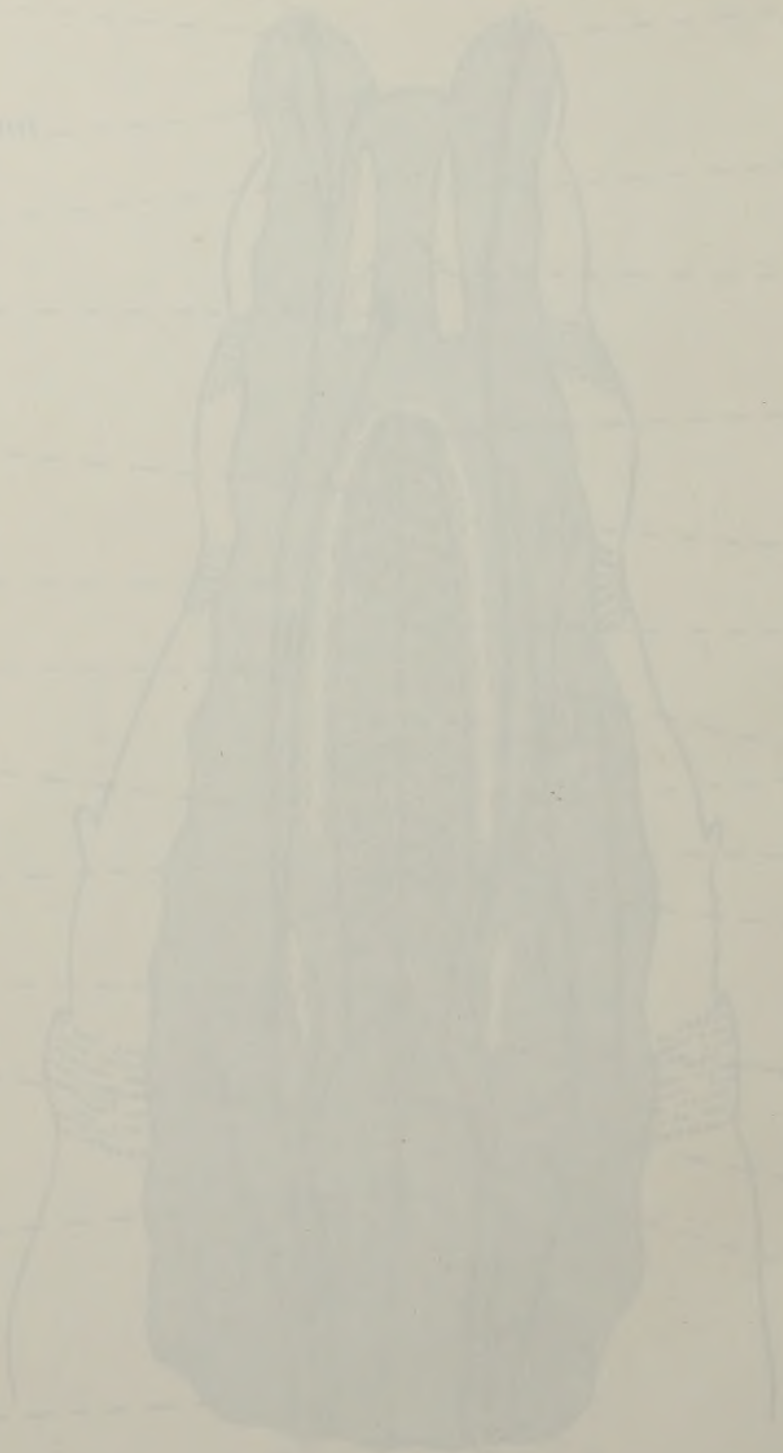


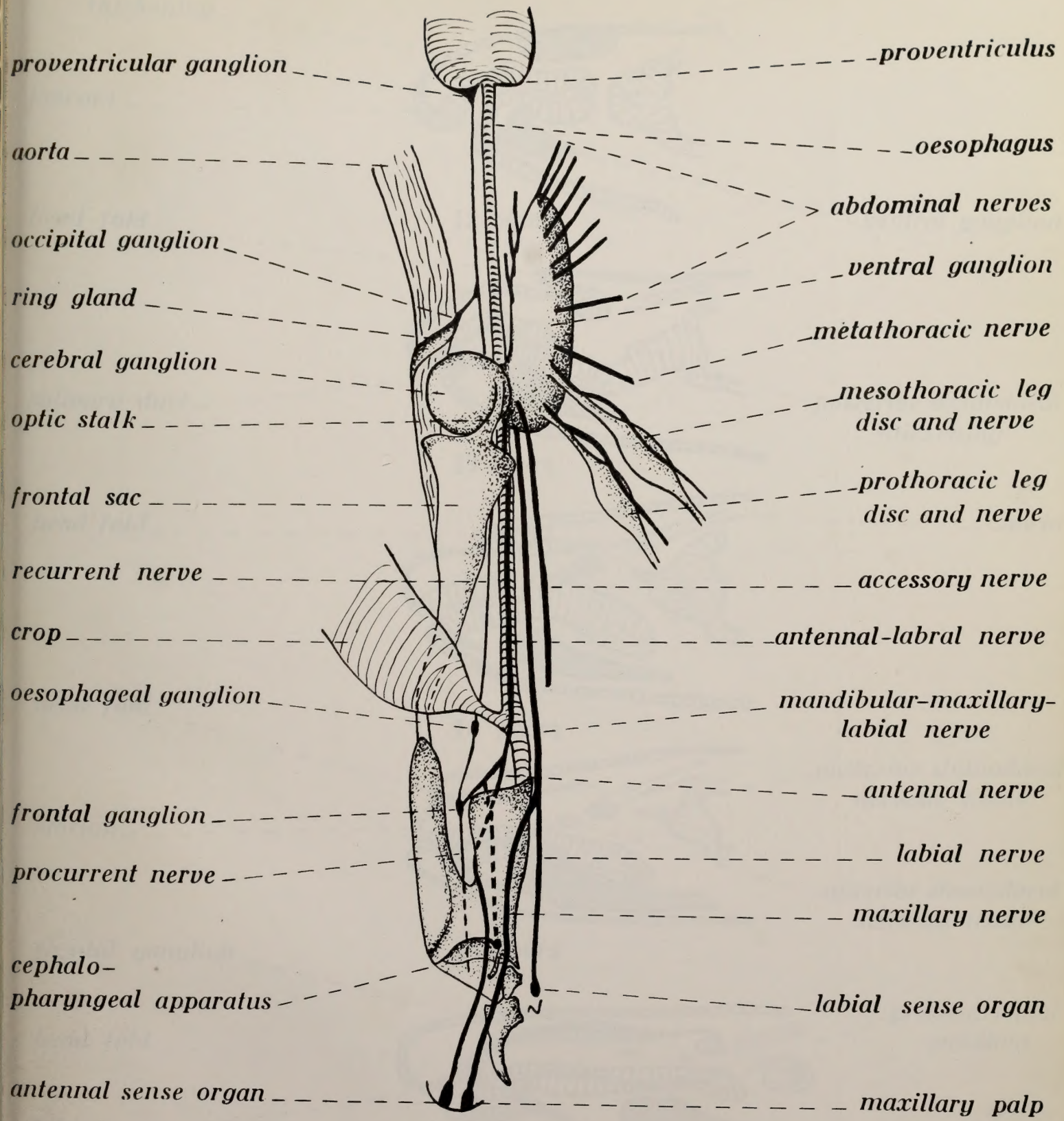
Figure 13 Details of cibarial-pharyngeal musculature, lateral aspect



Nerves to head of larva, dorsal aspect, semidiagrammatic

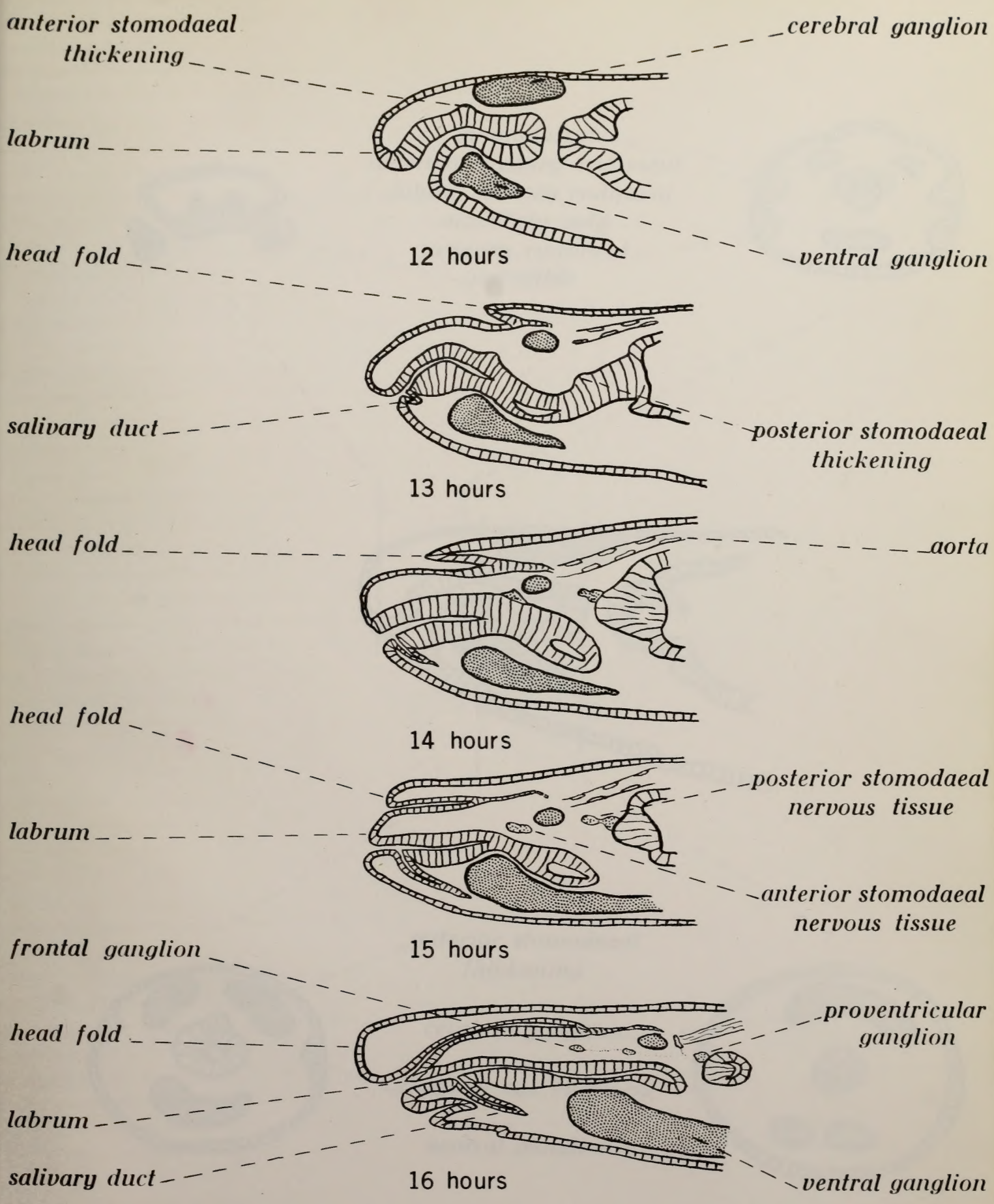
Figure 14





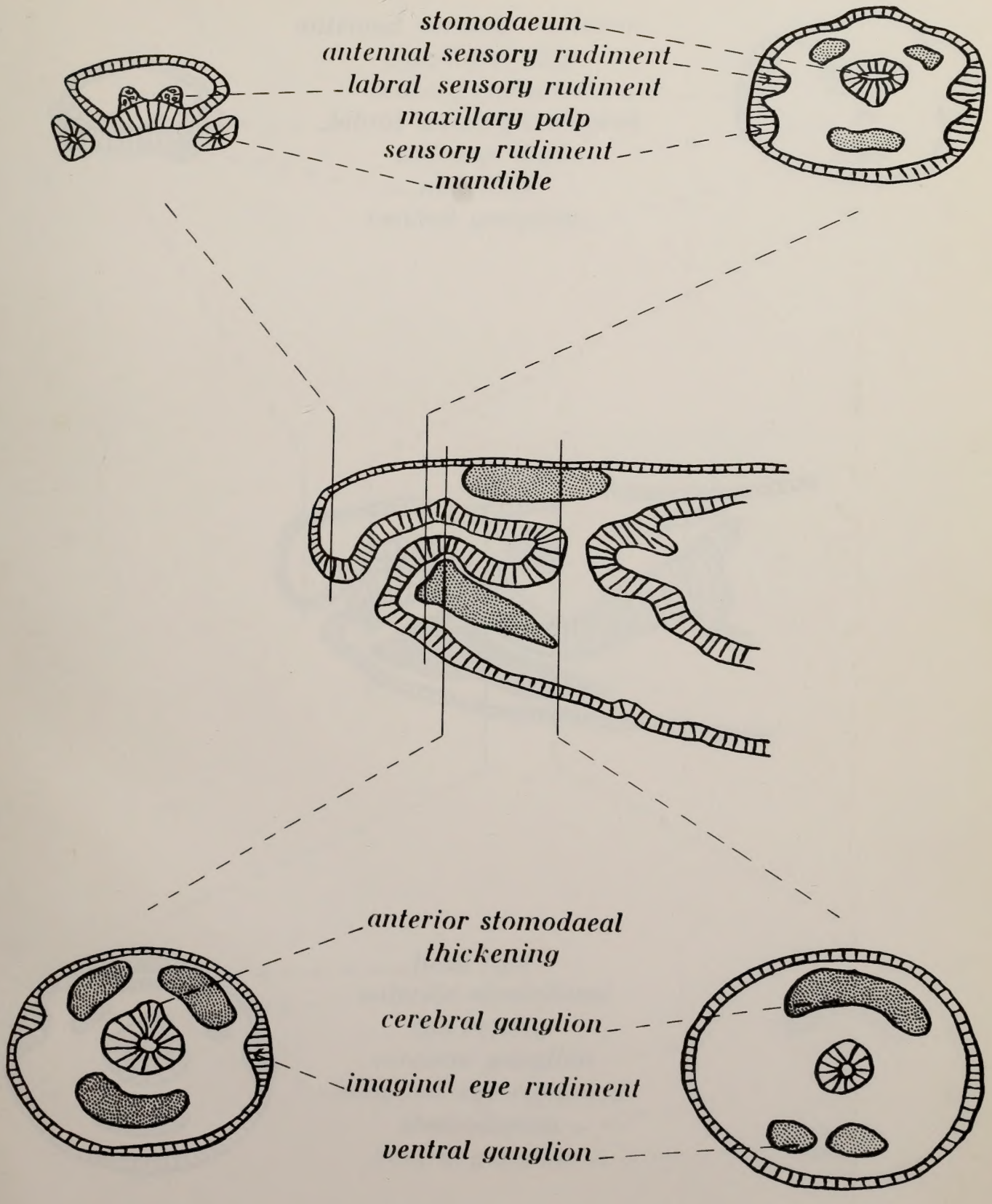
Nervous system of larva, lateral aspect, semidiagrammatic

Figure 15



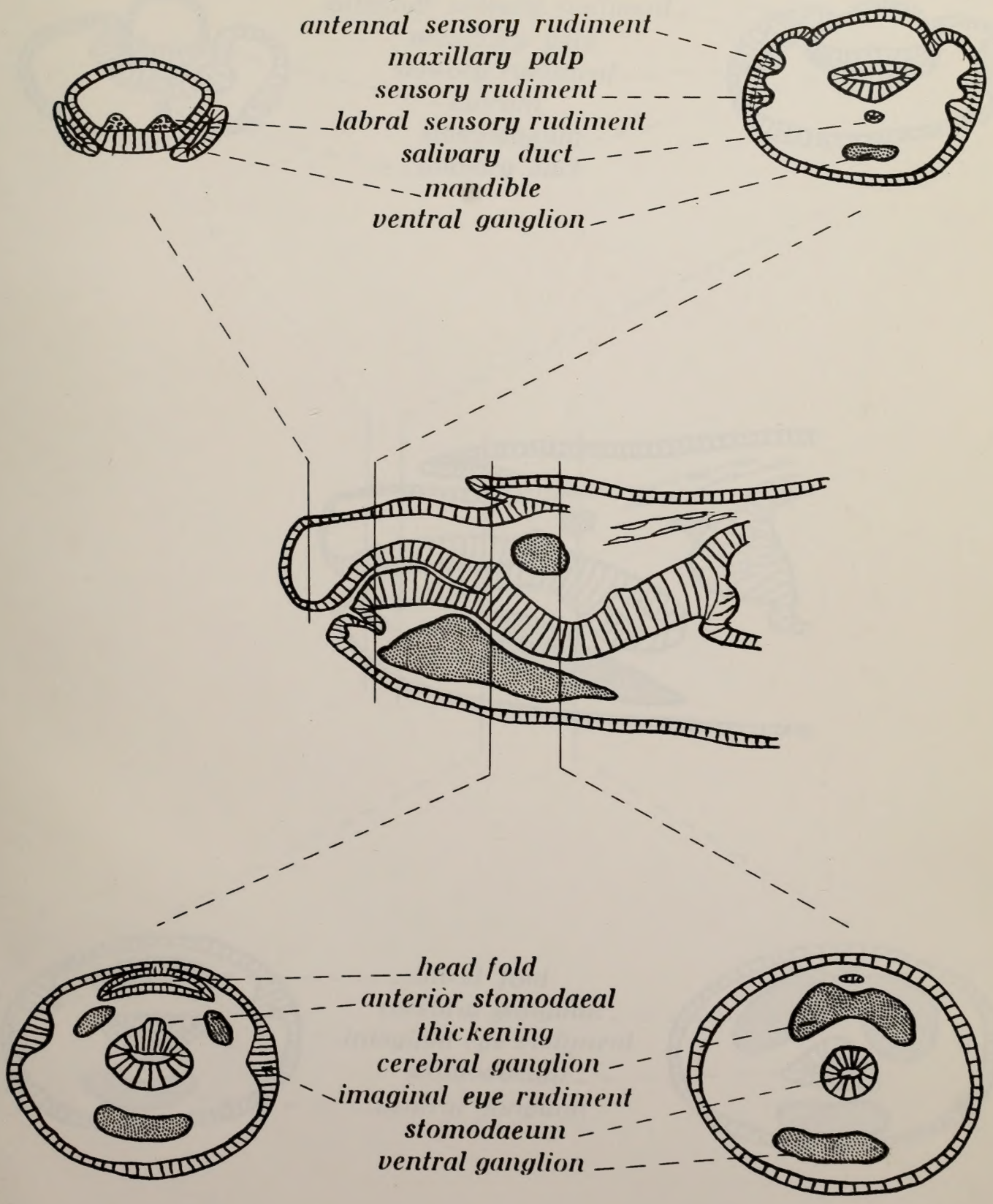
Development of stomodaeal nervous system and head fold, median longitudinal sections, 100 X

Figure 16



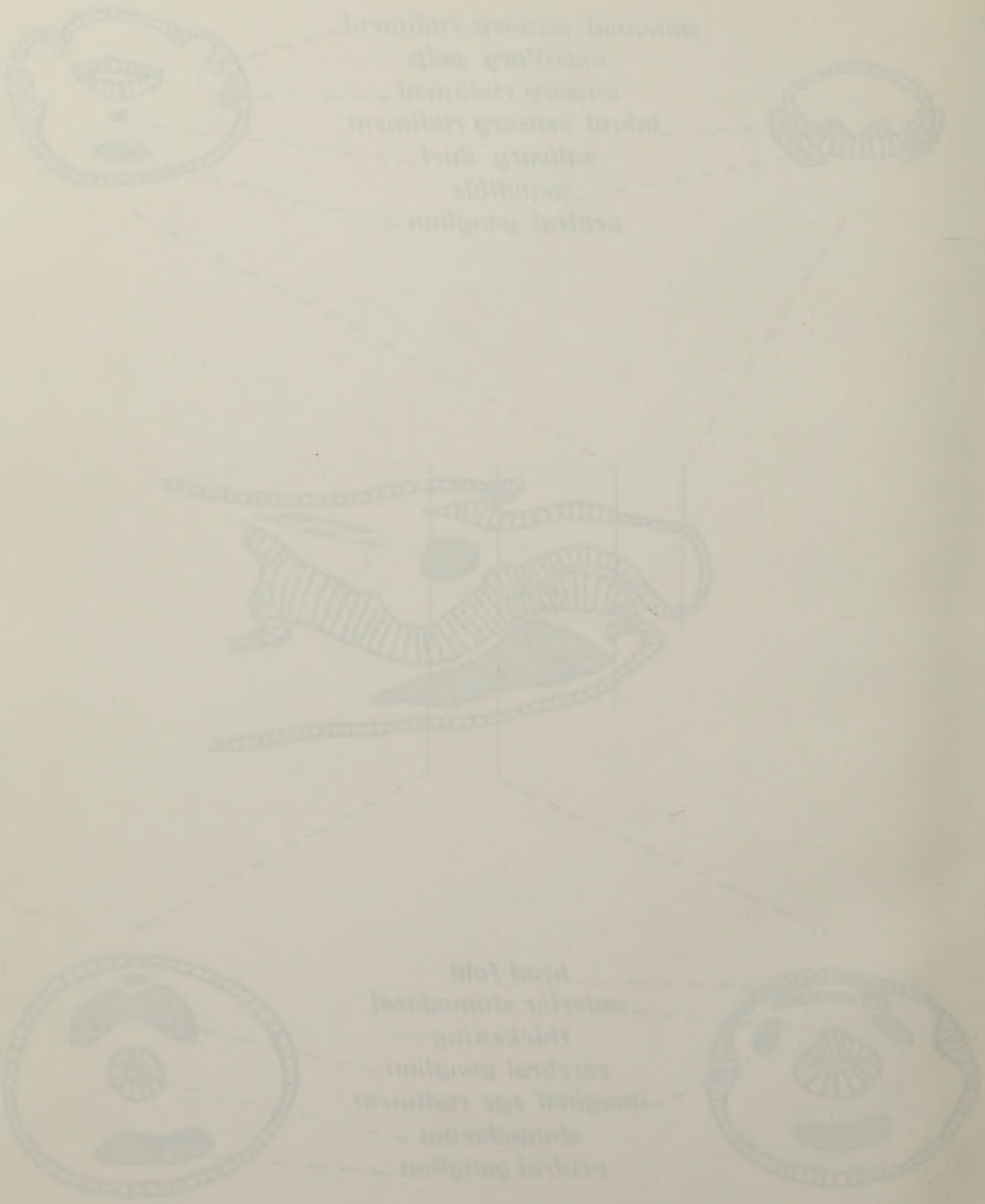
12 hour embryo, median longitudinal and cross sections, 150X

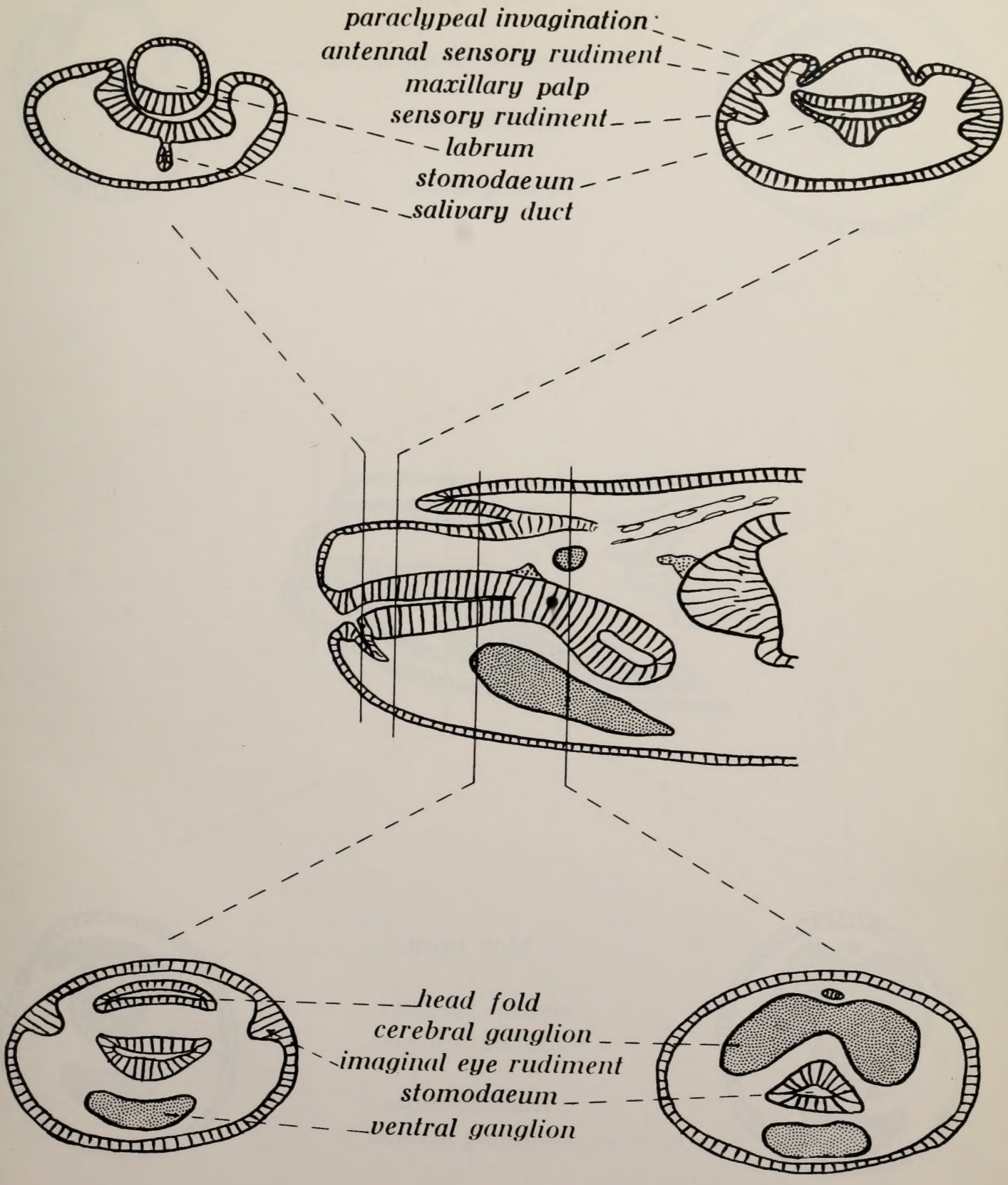
Figure 17



13 hour embryo, median longitudinal and cross sections, 150X

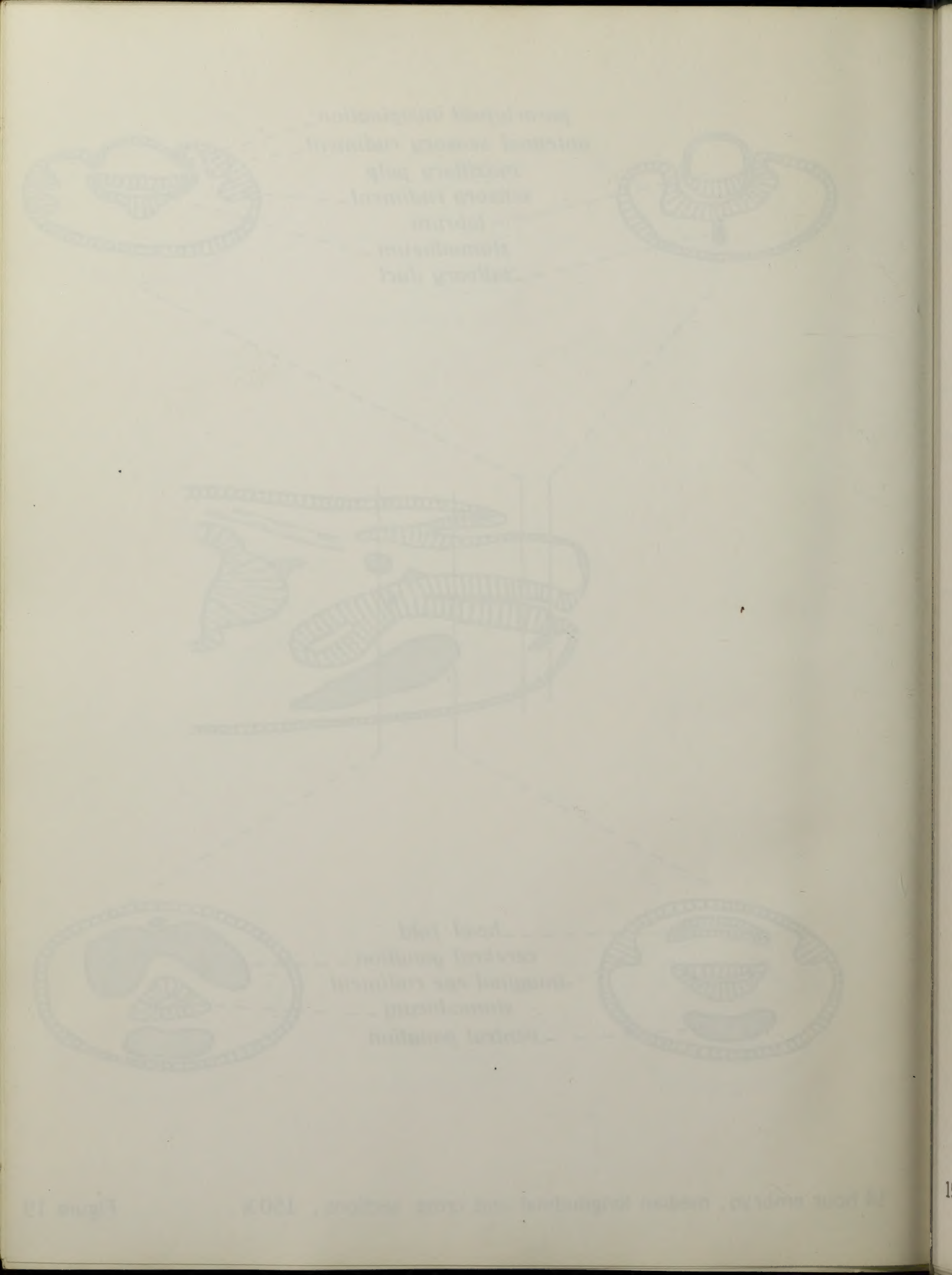
Figure 18

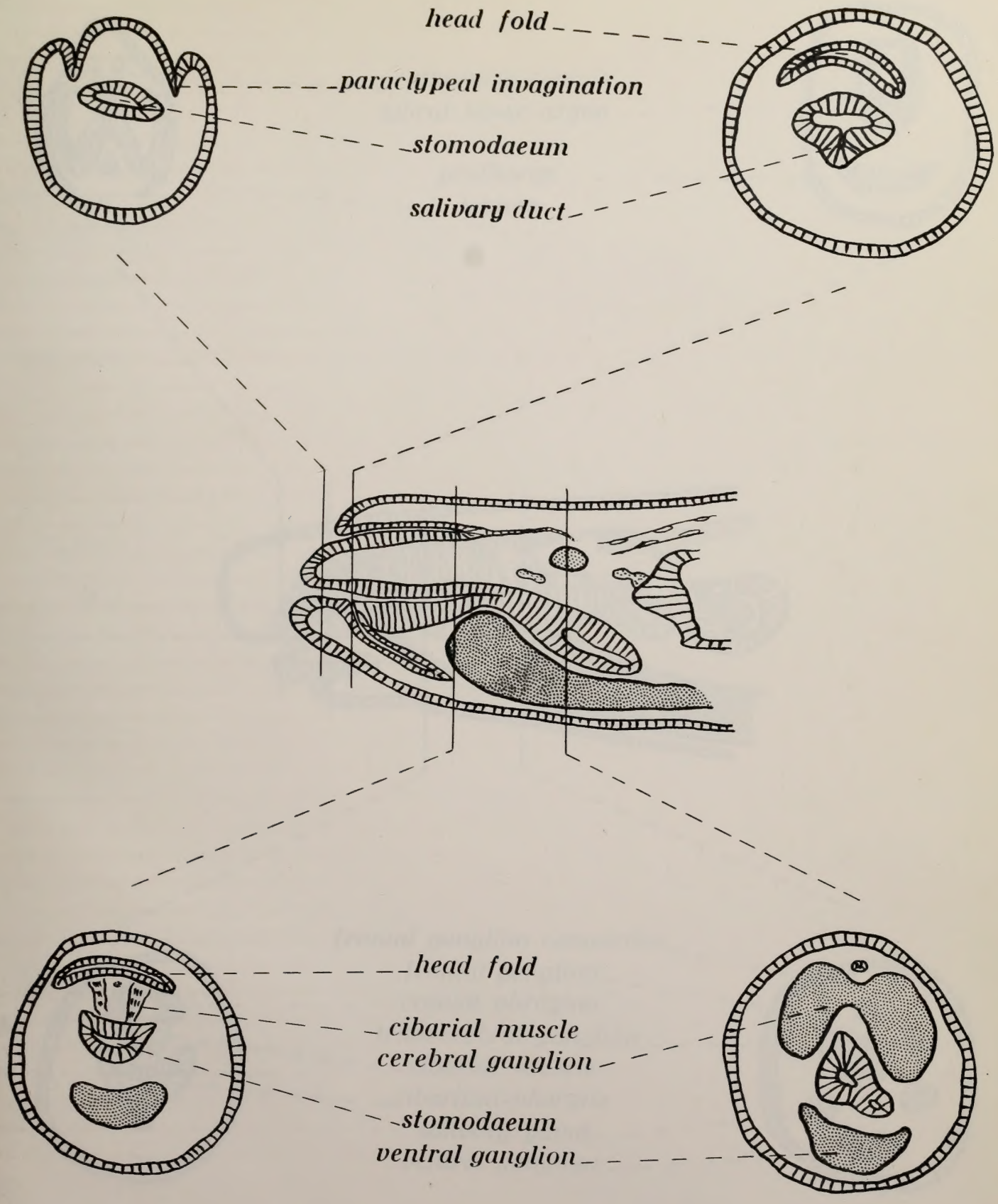




14 hour embryo, median longitudinal and cross sections, 150 X

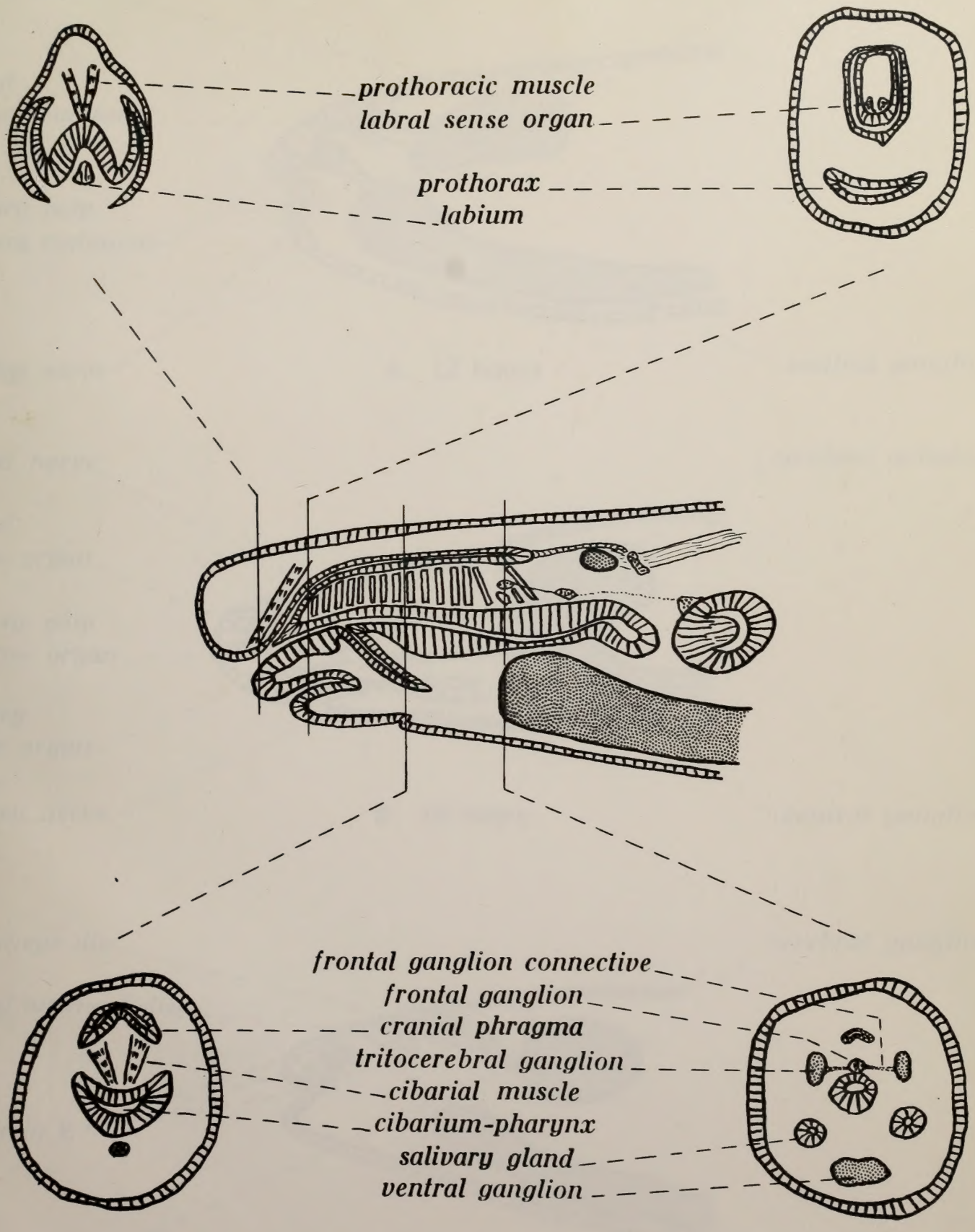
Figure 19





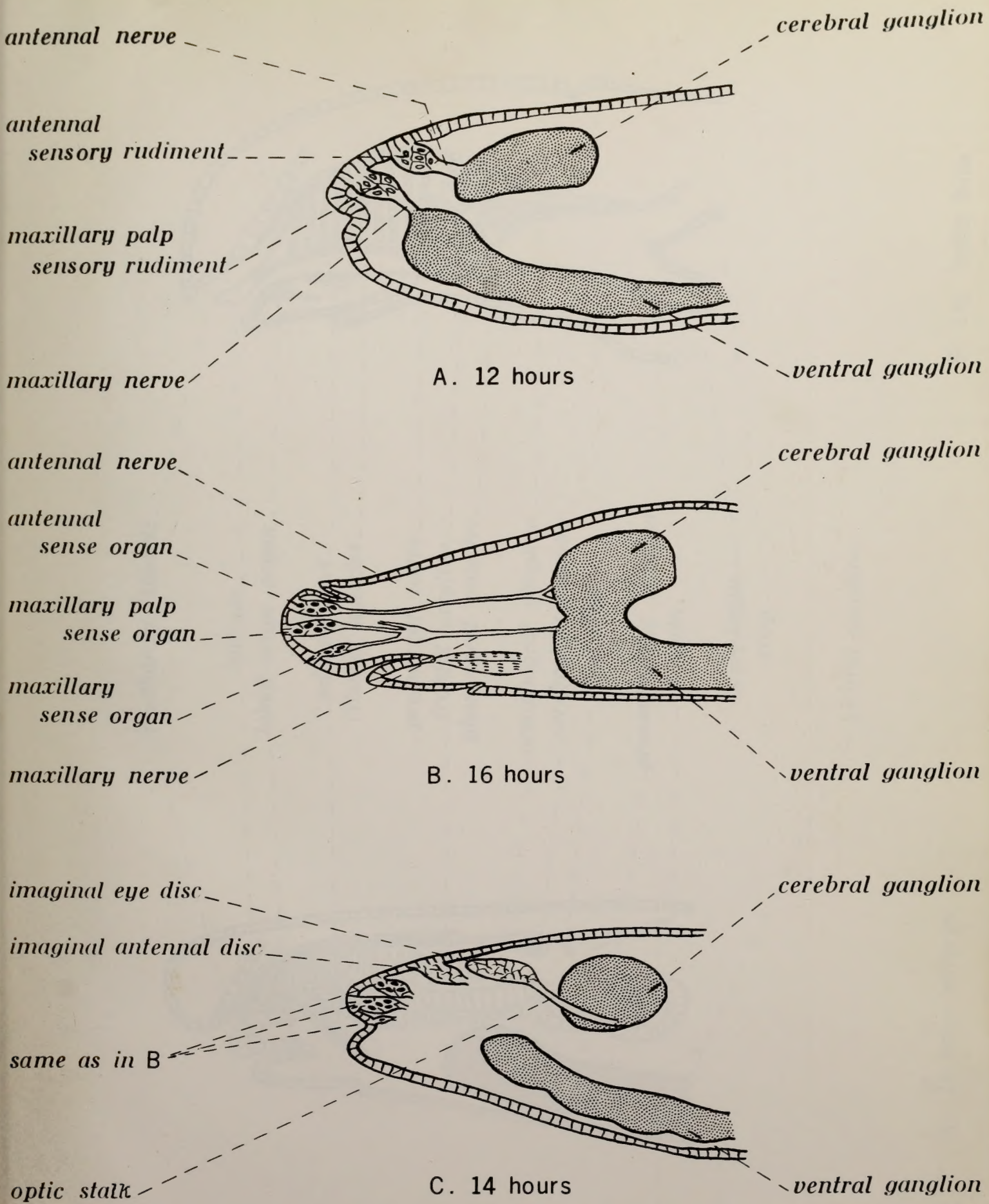
15 hour embryo, median longitudinal and cross sections, 150X

Figure 20



16 hour embryo, median longitudinal and cross sections, 150X

Figure 21



Development of sense organs, longitudinal sections, 150X

Figure 22

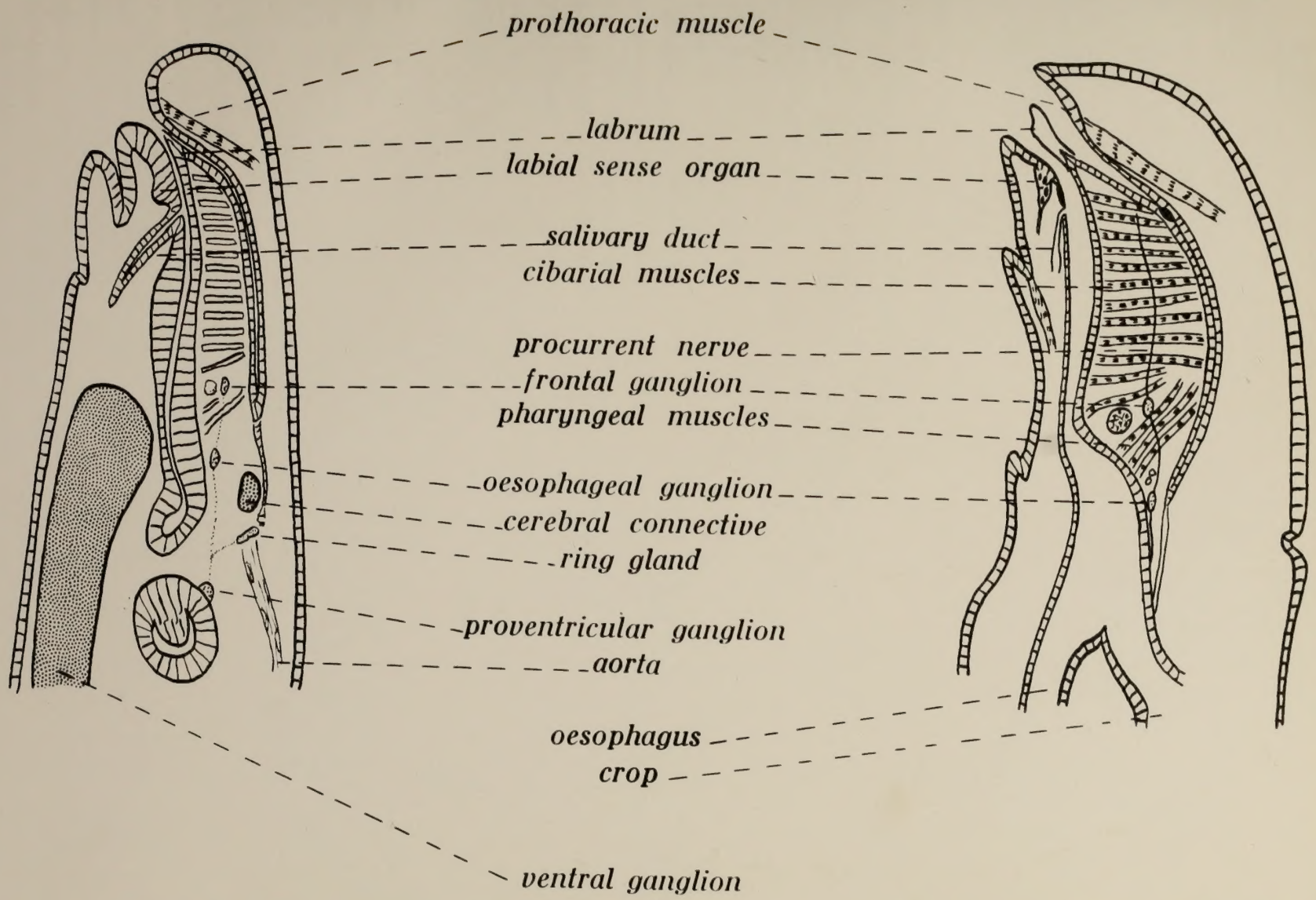


Figure 23 Head of larva and embryo, median longitudinal sections, 150X

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